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(54) Title: MAMMALIAN TRANSFORMING GROWTH FACTOR BETA – 9 (ZTGFSS9)

(57) Abstract

Novel mammalian Ztgfβ–9 polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods including antibodies and anti–idiotypic antibodies.

MAMMALIAN TRANSFORMING GROWTH FACTOR BETA - 9

BACKGROUND OF THE INVENTION

Proper control of the opposing processes of cell proliferation versus terminal differentiation and apoptotic programmed cell death is an important aspect of normal development and homeostasis, Raff, M.C., *Cell*, *86*:173-175 (1996), and has been found to be altered in many human diseases. See, for example, Sawyers, C.L. *et al.*, *Cell*, *64*:337-350 (1991); Meyaard, L. *et al.*, *Science*, *257*:217-219 (1992); Guo, Q. *et al.*, *Nature Med.*, *4*:957-962 (1998); Barinaga, M. *Science*, *273*:735-737 (1996); Solary, E. *et al.*, *Eur. Respir. J.*, *9*:1293-1305 (1996); Hamet, P. *et al.*, *J. Hypertension*, *14*:S65-S70, (1996); Roy, N. *et al. Cell*, *80*:167-178 (1995); and Ambrosini, G., *Nature Med.*, *8*:917-921 (1997). Much progress has been made towards understanding the regulation of this balance. For example, signaling cascades have been elucidated through which extracellular stimuli, such as growth factors, peptide hormones, and cell-cell interactions control the commitment of precursor cells to specific lineages and their subsequent proliferative expansion, Morrison, S.J. *et al.*, *Cell*, *88*:287-298 (1997). Further, it has been found that cell cycle exit and terminal differentiation are coupled in most cell types. See, for example, Coppola, J.A. *et al. Nature*, *320*:760-763 (1986); Freytag, S.O., *Mol. Cell. Biol.* *8*:1614-1624 (1988); Lee, E.Y. *et al.*, *Genes Dev.*, *8*:2008-2021 (1994); Morgenbesser, S.D., *et al.*, *Nature*, *371*:72-74 (1994); Casaccia-Bonnefil, P. *et al.*, *Genes Dev.*, *11*:2335-2346 (1996); Zacksenhaus, E. *et al.*, *Genes Dev.*, *10*:3051-3064 (1996); and Zhang, P. *et al.*, *Nature*, *387*:151-158 (1997). Apoptosis (programmed cell death) also plays an important role in many developmental and homeostatic processes, Raff, M.C.,

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Nature, *356*:397-400 (1992), and is often coordinately
regulated with terminal differentiation, Jacobsen, K.A. *et
al.*, *Blood*, *84*:2784-2794 (1994); Yan, Y. *et al.*, *Genes
Dev.*, *11*:973-983 (1997). Hence, it appears that the cell

5   type of individual lineages, tissues, organs, or even
entire multicellular organisms is the result of a finely
tuned balance between increased cell production due to
proliferation, and decreased numbers of cells resulting
from terminal differentiation and apoptosis. This balance

10   is most likely regulated coordinately by the convergence
of multiple regulatory pathways. The identification of
novel members of such networks can provide important
insights into both normal cellular processes as well as
the etiology and treatment of human disease states.

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Interleukin 17 (IL-17) is a cytokine which has been
implicated as an important regulator of the immune system,
Spriggs, M.K., *J. Clinical Immunology*, *17*:366-369 (1997),
Broxmeyer, H.E., *J. Experimental Medicine*, *183*:2411-2415

20   (1996), Yao, Z., *et al.*, *J. Immunology*, *155*:5483-
5486(1995), Yao, Z., *et al.*, *Immunity*, *3*:811-821 (1995).
Human IL-17 is almost exclusively produced by activated
CD4+ memory T cells(however, in mice,CD4-/CD8- T cells
also express IL-17), Aarvak, T., *et al.*, *J. Immunology*,

25   *162*:1246-1251 (1999), Kennedy, J., *et al.*, *J. Interferon
Cytokine Research*,*16*:611-617 (1996). In contrast, the IL-
17 receptor (IL-17R) appears to be ubiquitously expressed,
Yao, Z., et al., *Immunity*, *3*:811-821 (1995). IL-17 induces
the secretion of IL-6, IL-8, monocyte chemotactic peptide-

30   1 and G-CSF from a variety of different stromal cell
types, but has no effect on cytokine production by
lymphoid cells, Teunissen, M.B.M., *J. Investigative
Dermatology*, *111*:645-649 (1998), Jovanovic, D.V., *et al.*,
J. Immunology, *160*:3513-3521 (1998), Chabaud, M., *et al.*,

35   *J. Immunology*, *161*:409-414 (1998), Cai, X.-Y., *et al.*,
Immunology Letters, *62*:51-58 (1998), Fossiez, F., *et al.*,
J. Experimental Medicine, *183*:2593-2603 (1996). IL-17

also enhances the expression of ICAM-1 adhesion molecules
on fibroblasts, and can stimulate granulopoiesis,
Schwarzenberger P., et al., J. Immunology,
161:6383-9 (1998). . Taken together, these observations
5   have suggested that IL-17 functions as a pro-inflammatory
cytokine. IL-17 also promotes dendritic cell
differentiation, osteoclastogenesis, can induce nitric
oxide production in human osteoarthritis cartilage, and is
present in synovial fluids from patients with rheumatoid
10 arthritis, Antonysamy, M.A., et al., J. Immunology,
162:577-584 (1999), Kotake, S., et al., J. Clinical
Investigation, 103:1345-1352, (1999), Attur, M.G., et al.,
Arthritis & Rheumatism, 40:1050-1053 (1997). Blocking IL-
17 with a soluble IL-17R protein was found to suppress
15 cardiac allograft rejection, which correlated with
increased IL-17 mRNA in kidney biopsies from humans
undergoing renal allograft rejection, Antonysamy, M.A., et
al., J. Immunology, 162:577-584 (1999). Increased IL-17
mRNA expression is also observed in humans with multiple
20 sclerosis, Matusevicius, D. et al., Multiple Sclerosis,
5:101-104 (1999). Further, IL-17 can promote
tumorigenicity of human cervical tumors in nude mice,
Tartour, E. et al., Cancer Res., 59:3698-36704 (1999).
Hence, IL-17 appears to play an essential role in
25 regulating the immune system and inflammatory processes.

       Thus, there is a continuing need to discover new
proteins involved with proliferation, differentiation, and
30 apoptotic pathways. The in vivo activities of both
inducers and inhibitors of these pathways illustrates the
enormous clinical potential of, and need for, novel
proliferation, differentiation, and apoptotic proteins,
their agonists and antagonists. There is also a need to
35 discover new agents which have anti-viral activity.

SUMMARY OF THE INVENTION

        The present invention addresses this need by
providing a novel anti-viral polypeptide called
5   transforming growth factor beta-9,hereinafter referred to
as Ztgfβ-9, and related compositions and methods. This
polypeptide has anti-viral activity as disclosed in
Example 10 below. It may also be used to regulate the
proliferation, differentiation and apoptosis of neurons
10 glial cells, lymphocytes, hematopoietic cells and stromal
cells.

        Thus, one aspect of the present invention
provides for an isolated Ztgfβ-9 polypeptide and
15 polynucleotide. The human sequences are defined by SEQ ID
NOs: 1 and 2.

        The nucleotide sequence of SEQ ID NO:1 contains
an open reading frame encoding a polypeptide of about 202
20 amino acids with the initial Met as shown in SEQ ID NO:1
and SEQ ID NO:2. A predicted signal sequence is comprised
of amino acid residues 1, a methionine extending to and
includes amino acid residue 15, an alanine. Thus a mature
sequence excluding the signal sequence extends from amino
25 acid residue 16, an alanine, to and including amino acid
residue 202 a proline, of SEQ ID NO:2. This mature
sequence is also represented by SEQ ID NO:3. In an
alternative embodiment the signal sequence extends to and
includes amino acid residue 16, an alanine. This produces
30 a mature sequence which extends from amino acid 17, a
glycine, to and including amino acid residue 202, a
proline, of SEQ ID NO:2. This mature sequence is also
represented by SEQ ID NO:4. In another alternative
embodiment, the signal sequence extends to and includes
35 amino acid residue 17, a glycine. This results in a mature
sequence which extends from amino acid residue 18, an
alanine, to and including amino acid residue 202, a

proline, of SEQ ID NO:2. This mature sequence is further
represented by SEQ ID NO:5. Another variant of Ztgfβ-9 is
disclosed by SEQ ID NOs: 16 and 17. The mature sequence
extends from amino acid residue 23, an alanine, to and
5 including amino acid residue 209, a proline. The mature
sequence is also defined by SEQ ID NO:18.

Murine Ztgfβ-9 is defined by SEQ ID NOs: 8 and
9. The signal sequence extends from the methionine at
10 position 1 through the alanine at position 22. Thus the
mature sequence extends from the alanine at position 23 of
SEQ ID NO:9 through the arginine at position 205. The
mature sequence is further represented by SEQ ID NO:12.

15 An additional embodiment of the present
invention relates to a peptide or polypeptide which has
the amino acid sequence of an epitope-bearing portion of a
Ztgfβ-9 polypeptide having an amino acid sequence
described above. Peptides or polypeptides having the amino
20 acid sequence of an epitope-bearing portion of a Ztgfβ-9
polypeptide of the present invention include portions of
such polypeptides with at least nine, preferably at least
15 and more preferably at least 30 to 50 amino acids,
although epitope-bearing polypeptides of any length up to
25 and including the entire amino acid sequence of a
polypeptide of the present invention described above are
also included in the present invention. Examples of such
epitope-bearing polypeptides are SEQ ID NOs: 13, 14, 15,
19, 20, 21 and 22. Also claimed are any of these
30 polypeptides that are fused to another polypeptide or
carrier molecule. Also claimed is an isolated nucleic acid
which encodes an epitope-bearing portion of a Ztgfβ-9
polypeptide.

35 The present invention is further comprised of an
isolated peptide or polypeptide of the above-described
peptides or polypeptides having an amino acid sequence

modified by addition, deletion and/or replacement of one
or more amino acid residues and which maintains the
biological activity of said peptide or polypeptide.

5   Within a further aspect of the invention there
is provided a chimeric polypeptide consisting essentially
of a first portion and a second portion joined by a
peptide bond. The first portion of the chimeric
polypeptide consists essentially of (a) a Ztgfβ-9
10   polypeptide as described above (b) allelic variants of the
polypeptides described above. The second portion of the
chimeric polypeptide consists essentially of another
polypeptide such as an affinity tag. Within one
embodiment the affinity tag is an immunoglobulin F_C
15   polypeptide. The invention also provides expression
vectors encoding the chimeric polypeptides and host cells
transfected to produce the chimeric polypeptides.

          Another aspect of the present invention provides
20   for isolated nucleic acid molecules comprising a
polynucleotide selected from the group consisting of: (a)
a nucleotide sequence encoding the Ztgfβ-9 polypeptides
described above; and (b) a nucleotide sequence
complementary to any of the nucleotide sequences in (a).
25

          Further embodiments of the invention include
isolated nucleic acid molecules that comprise a
polynucleotide having a nucleotide sequence at least 90%
homologous, and more preferably 95%, 97%, 98%, or 99%
30   homologous to any of the nucleotide sequences in (a) or
(b) above, or a polynucleotide which hybridizes under
stringent hybridization conditions to a polynucleotide
having a nucleotide sequence of (a) or (b) above.

35   Further embodiments of the invention include
isolated polypeptides having an amino acid sequence that
is at least 90% identical, and more preferably 95%, 97%,

98%, or 99% identical to any of the Ztgfβ-9 polypeptides and polynucleotides which encode these polypeptides.

Within another aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding a polypeptide described above, and (c) a transcription terminator, wherein the promoter, DNA segment, and terminator are operably linked.

Within a third aspect of the invention there is provided a cultured eukaryotic cell into which has been introduced an expression vector as disclosed above, wherein said cell expresses a protein polypeptide encoded by the DNA segment.

In another embodiment of the present invention is an isolated antibody that binds specifically to a Ztgfβ-9 polypeptide described above. Also claimed is a method for producing antibodies which bind to a Ztgfβ-9 polypeptide comprising inoculating a mammal with a Ztgfβ-9 polypeptide or Ztgfβ-9 epitope-bearing polypeptide so that the mammal produces antibodies to the polypeptide; and isolating said antibodies.

These and other aspects of the invention will become evident upon reference to the following detailed description.

DETAILED DESCRIPTION OF THE INVENTION

The teachings of all of the references cited herein are incorporated in their entirety herein by reference.

In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

5   As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of

10   ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., α-enantiomeric forms of naturally-

15   occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups,

20   amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety

25   include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate,

30   phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-

35   occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a nucleic acid molecule that has a contiguous stretch of identical or complementary sequence to another nucleic acid molecule. Contiguous sequences are said to "overlap" a given stretch of a nucleic acid molecule either in their entirety or along a partial stretch of the nucleic acid molecule.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than

the complete DNA molecule of a chromosome from that species.

A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

"Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

"Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee et al., *Mol. Endocrinol.* 7:551 (1993)), cyclic AMP response elements (CREs), serum response elements (SREs; Treisman, *Seminars in Cancer*

Biol. 1:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly *et al.*, *J. Biol. Chem.* 267:19938 (1992)), AP2 (Ye *et al.*, *J. Biol. Chem.* 269:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr.* 3:253 (1993)) and octamer factors (see, in general, Watson *et al.*, eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J.* 303:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner. For example, the Ztgfβ-9 regulatory element preferentially induces gene expression in brain, spinal cord, heart, skeletal muscle, stomach, pancreas, adrenal gland, salivary gland, liver, small intestine, bone marrow, thymus, spleen, lymph node, heart, thyroid, trachea, testis, ovary and placenta.

An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

"Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (i.e., endogenous DNA) so long as that host DNA is combined with non-host DNA (i.e., exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone

structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

An "integrated genetic element" is a segment of DNA that has been incorporated into a chromosome of a host cell after that element is introduced into the cell through human manipulation. Within the present invention, integrated genetic elements are most commonly derived from linearized plasmids that are introduced into the cells by electroporation or other techniques. Integrated genetic elements are passed from the original host cell to its progeny.

A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, that has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a

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core promoter are operably linked if the regulatory element
modulates the activity of the core promoter.

5   A "recombinant host" is a cell that contains a
heterologous nucleic acid molecule, such as a cloning
vector or expression vector. In the present context, an
example of a recombinant host is a cell that produces
Ztgfβ-9 from an expression vector. In contrast, Ztgfβ-9
can be produced by a cell that is a "natural source" of
10   Ztgfβ-9, and that lacks an expression vector.

"Integrative transformants" are recombinant host
cells, in which heterologous DNA has become integrated
into the genomic DNA of the cells.

15
A "fusion protein" is a hybrid protein expressed
by a nucleic acid molecule comprising nucleotide sequences
of at least two genes. For example, a fusion protein can
comprise at least part of a Ztgfβ-9 polypeptide fused with
20   a polypeptide that binds an affinity matrix. Such a
fusion protein provides a means to isolate large
quantities of Ztgfβ-9 using affinity chromatography.

The term "receptor" denotes a cell-associated
25   protein that binds to a bioactive molecule termed a
"ligand." This interaction mediates the effect of the
ligand on the cell. Receptors can be membrane bound,
cytosolic or nuclear; monomeric (e.g., thyroid stimulating
hormone receptor, beta-adrenergic receptor) or multimeric
30   (e.g., PDGF receptor, growth hormone receptor, IL-3
receptor, GM-CSF receptor, G-CSF receptor, erythropoietin
receptor and IL-6 receptor). Membrane-bound receptors are
characterized by a multi-domain structure comprising an
extracellular ligand-binding domain and an intracellular
35   effector domain that is typically involved in signal
transduction. In certain membrane-bound receptors, the
extracellular ligand-binding domain and the intracellular

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effector domain are located in separate polypeptides that
comprise the complete functional receptor.

5   In general, the binding of ligand to receptor
results in a conformational change in the receptor that
causes an interaction between the effector domain and
other molecule(s) in the cell, which in turn leads to an
alteration in the metabolism of the cell. Metabolic events
that are often linked to receptor-ligand interactions
10   include gene transcription, phosphorylation,
dephosphorylation, increases in cyclic AMP production,
mobilization of cellular calcium, mobilization of membrane
lipids, cell adhesion, hydrolysis of inositol lipids and
hydrolysis of phospholipids.

15

         The term "secretory signal sequence" denotes a
DNA sequence that encodes a peptide (a "secretory
peptide") that, as a component of a larger polypeptide,
directs the larger polypeptide through a secretory pathway
20   of a cell in which it is synthesized. The larger
polypeptide is commonly cleaved to remove the secretory
peptide during transit through the secretory pathway.

         An "isolated polypeptide" is a polypeptide that
25   is essentially free from contaminating cellular
components, such as carbohydrate, lipid, or other
proteinaceous impurities associated with the polypeptide
in nature. Typically, a preparation of isolated
polypeptide contains the polypeptide in a highly purified
30   form, i.e., at least about 80% pure, at least about 90%
pure, at least about 95% pure, greater than 95% pure, or
greater than 99% pure. One way to show that a particular
protein preparation contains an isolated polypeptide is by
the appearance of a single band following sodium dodecyl
35   sulfate (SDS)-polyacrylamide gel electrophoresis of the
protein preparation and Coomassie Brilliant Blue staining
of the gel. However, the term "isolated" does not exclude

the presence of the same polypeptide in alternative
physical forms, such as dimers or alternatively
glycosylated or derivatized forms.

5   The terms "amino-terminal or N-terminal" and
"carboxyl-terminal or C-terminal" are used herein to
denote positions within polypeptides. Where the context
allows, these terms are used with reference to a
particular sequence or portion of a polypeptide to denote
10   proximity or relative position. For example, a certain
sequence positioned carboxyl-terminal to a reference
sequence within a polypeptide is located proximal to the
carboxyl terminus of the reference sequence, but is not
necessarily at the carboxyl terminus of the complete
15   polypeptide.

          The term "expression" refers to the biosynthesis
of a gene product. For example, in the case of a
structural gene, expression involves transcription of the
20   structural gene into mRNA and the translation of mRNA into
one or more polypeptides.

          The term "splice variant" is used herein to
denote alternative forms of RNA transcribed from a gene.
25   Splice variation arises naturally through use of
alternative splicing sites within a transcribed RNA
molecule, or less commonly between separately transcribed
RNA molecules, and may result in several mRNAs transcribed
from the same gene. Splice variants may encode
30   polypeptides having altered amino acid sequence. The term
splice variant is also used herein to denote a polypeptide
encoded by a splice variant of an mRNA transcribed from a
gene.

35   As used herein, the term "immunomodulator"
includes cytokines, stem cell growth factors,

lymphotoxins, co-stimulatory molecules, hematopoietic factors, and synthetic analogs of these molecules.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than 10^9 M^{-1}.

An "anti-idiotype antibody" is an antibody that binds with the variable region domain of an immunoglobulin. In the present context, an anti-idiotype antibody binds with the variable region of an anti- Ztgfβ-9 antibody, and thus, an anti-idiotype antibody mimics an epitope of Ztgfβ-9.

An "antibody fragment" is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, a Ztgfβ-9 monoclonal antibody fragment binds with an epitope of Ztgfβ-9.

The term "antibody fragment" also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are

connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

A "chimeric antibody" is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

"Humanized antibodies" are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred from heavy and light variable chains of the murine immunoglobulin into a human variable domain.

As used herein, a "therapeutic agent" is a molecule or atom which is conjugated to an antibody moiety to produce a conjugate which is useful for therapy. Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

A "detectable label" is a molecule or atom which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag.

Affinity tags include a poly-histidine tract, protein A
(Nilsson *et al.*, *EMBO J.* 4:1075 (1985); Nilsson *et al.*,
Methods Enzymol. 198:3 (1991)), glutathione S transferase
(Smith and Johnson, *Gene* 67:31 (1988)), Glu-Glu affinity

5 tag (Grussenmeyer *et al.*, *Proc. Natl. Acad. Sci. USA*
82:7952 (1985)), substance P, FLAG peptide (Hopp *et al.*,
Biotechnology 6:1204 (1988)), streptavidin binding
peptide, or other antigenic epitope or binding domain.
See, in general, Ford *et al.*, *Protein Expression and*

10 *Purification* 2:95 (1991). DNAs encoding affinity tags are
available from commercial suppliers (*e.g.*, Pharmacia
Biotech, Piscataway, NJ).

A "naked antibody" is an entire antibody, as

15 opposed to an antibody fragment, which is not conjugated
with a therapeutic agent. Naked antibodies include both
polyclonal and monoclonal antibodies, as well as certain
recombinant antibodies, such as chimeric and humanized
antibodies.

20

As used herein, the term "antibody component"
includes both an entire antibody and an antibody fragment.

An "immunoconjugate" is a conjugate of an

25 antibody component with a therapeutic agent or a
detectable label.

As used herein, the term "antibody fusion
protein" refers to a recombinant molecule that comprises

30 an antibody component and a therapeutic agent. Examples
of therapeutic agents suitable for such fusion proteins
include immunomodulators ("antibody-immunomodulator fusion
protein") and toxins ("antibody-toxin fusion protein").

35 A "tumor associated antigen" is a protein
normally not expressed, or expressed at lower levels, by a
normal counterpart cell. Examples of tumor associated

antigens include alpha-fetoprotein, carcinoembryonic
antigen, and Her-2/neu. Many other illustrations of tumor
associated antigens are known to those of skill in the
art. See, for example, Urban *et al., Ann. Rev. Immunol.*
5 *10*:617 (1992).

        As used herein, an "infectious agent" denotes
both microbes and parasites. A "microbe" includes
viruses, bacteria, rickettsia, mycoplasma, protozoa, fungi
10 and like microorganisms. A "parasite" denotes infectious,
generally microscopic or very small multicellular
invertebrates, or ova or juvenile forms thereof, which are
susceptible to immune-mediated clearance or lytic or
phagocytic destruction, such as malarial parasites,
15 spirochetes, and the like.

        ' An "infectious agent antigen" is an antigen
associated with an infectious agent.

20   A "target polypeptide" or a "target peptide" is
an amino acid sequence that comprises at least one
epitope, and that is expressed on a target cell, such as a
tumor cell, or a cell that carries an infectious agent
antigen. T cells recognize peptide epitopes presented by
25 a major histocompatibility complex molecule to a target
polypeptide or target peptide and typically lyse the
target cell or recruit other immune cells to the site of
the target cell, thereby killing the target cell.

30   An "antigenic peptide" is a peptide which will
bind a major histocompatibility complex molecule to form
an MHC-peptide complex which is recognized by a T cell,
thereby inducing a cytotoxic lymphocyte response upon
presentation to the T cell. Thus, antigenic peptides are
35 capable of binding to an appropriate major
histocompatibility complex molecule and inducing a
cytotoxic T cells response, such as cell lysis or specific

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cytokine release against the target cell which binds or
expresses the antigen. The antigenic peptide can be bound
in the context of a class I or class II major
histocompatibility complex molecule, on an antigen
5   presenting cell or on a target cell.

        In eukaryotes, RNA polymerase II catalyzes the
transcription of a structural gene to produce mRNA. A
nucleic acid molecule can be designed to contain an RNA
10 polymerase II template in which the RNA transcript has a
sequence that is complementary to that of a specific mRNA.
The RNA transcript is termed an "anti-sense RNA" and a
nucleic acid molecule that encodes the anti-sense RNA is
termed an "anti-sense gene." Anti-sense RNA molecules are
15 capable of binding to mRNA molecules, resulting in an
inhibition of mRNA translation or mRNA degradation.

        An "anti-sense oligonucleotide specific for
$Ztgf\beta$-9" or a "$Ztgf\beta$-9 anti-sense oligonucleotide" is an
20 oligonucleotide having a sequence (a) capable of forming a
stable triplex with a portion of the $Ztgf\beta$-9 gene, or (b)
capable of forming a stable duplex with a portion of an
mRNA transcript of the $Ztgf\beta$-9 gene.

25   A "ribozyme" is a nucleic acid molecule that
contains a catalytic center. The term includes RNA
enzymes, self-splicing RNAs, self-cleaving RNAs, and
nucleic acid molecules that perform these catalytic
functions. A nucleic acid molecule that encodes a
30 ribozyme is termed a "ribozyme gene."

        An "external guide sequence" is a nucleic acid
molecule that directs the endogenous ribozyme, RNase P, to
a particular species of intracellular mRNA, resulting in
35 the cleavage of the mRNA by RNase P. A nucleic acid
molecule that encodes an external guide sequence is termed
an "external guide sequence gene."

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The term "variant human *Ztgfβ-9* gene" refers to
nucleic acid molecules that encode a polypeptide having an
amino acid sequence that is a modification of SEQ ID NO:2.
5   Such variants include naturally-occurring polymorphisms of
Ztgfβ-9 genes, as well as synthetic genes that contain
conservative amino acid substitutions of the amino acid
sequence of SEQ ID NO:2. Additional variant forms of
Ztgfβ-9 genes are nucleic acid molecules that contain
10 insertions or deletions of the nucleotide sequences
described herein. A variant Ztgfβ-9 gene can be
identified by determining whether the gene hybridizes with
a nucleic acid molecule having the nucleotide sequence of
SEQ ID NO:1, or its complement, under stringent
15 conditions.

Similarly, the term "variant murine *Ztgfβ-9*
gene" refers to nucleic acid molecules that encode a
polypeptide having an amino acid sequence that is a
20 modification of SEQ ID NO:9. A variant murine *Ztgfβ-9*
gene can be identified by determining whether the gene
hybridizes with a nucleic acid molecule having the
nucleotide sequence of SEQ ID NO:8, or its complement,
under stringent conditions.
25

Alternatively, variant *Ztgfβ-9* genes can be
identified by sequence comparison. Two amino acid
sequences have "100% amino acid sequence identity" if the
amino acid residues of the two amino acid sequences are
30 the same when aligned for maximal correspondence.
Similarly, two nucleotide sequences have "100% nucleotide
sequence identity" if the nucleotide residues of the two
nucleotide sequences are the same when aligned for maximal
correspondence. Sequence comparisons can be performed
35 using standard software programs such as those included in
the LASERGENE bioinformatics computing suite, which is
produced by DNASTAR (Madison, Wisconsin). Other methods

for comparing two nucleotide or amino acid sequences by
determining optimal alignment are well-known to those of
skill in the art (see, for example, Peruski and Peruski,
The Internet and the New Biology: Tools for Genomic and
5   *Molecular Research* (ASM Press, Inc. 1997), Wu et al.
(eds.), "Information Superhighway and Computer Databases
of Nucleic Acids and Proteins," in *Methods in Gene
Biotechnology*, pages 123-151 (CRC Press, Inc. 1997), and
Bishop (ed.), *Guide to Human Genome Computing*, 2nd Edition
10   (Academic Press, Inc. 1998)). Particular methods for
determining sequence identity are described below.

        Regardless of the particular method used to
identify a variant *Ztgfβ-9* gene or variant Ztgfβ-9
15   polypeptide, a variant gene or polypeptide encoded by a
variant gene is functionally characterized by either its
anti-viral or anti-proliferative activities, or by the
ability to bind specifically to an anti-Ztgfβ-9 antibody.

20   The term "allelic variant" is used herein to
denote any of two or more alternative forms of a gene
occupying the same chromosomal locus. Allelic variation
arises naturally through mutation, and may result in
phenotypic polymorphism within populations. Gene
25   mutations can be silent (no change in the encoded
polypeptide) or may encode polypeptides having altered
amino acid sequence. The term allelic variant is also used
herein to denote a protein encoded by an allelic variant
of a gene.
30

        The term "ortholog" denotes a polypeptide or
protein obtained from one species that is the functional
counterpart of a polypeptide or protein from a different
species. Sequence differences among orthologs are the
35   result of speciation.

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"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

5

Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated

10 value of X will be understood to be accurate to ±10%.

Nucleic acid molecules encoding a human or mouse *Ztgfβ-9* gene can be obtained by screening a human or mouse cDNA or genomic library using polynucleotide probes based

15 upon SEQ ID NO:1 or SEQ ID NO:8. These techniques are standard and well-established. As an illustration, a nucleic acid molecule that encodes a human *Ztgfβ-9* gene can be isolated from a human cDNA library. In this case, the first step would be to prepare the cDNA library by

20 isolating RNA from brain, spinal cord, heart, skeletal muscle, stomach, pancreas, adrenal gland, salivary gland, liver, small intestine, bone marrow, thymus, spleen, lymph node, heart, thyroid, trachea, testis, ovary or placental tissue, using methods well-known to those of skill in the

25 art. In general, RNA isolation techniques must provide a method for breaking cells, a means of inhibiting RNase-directed degradation of RNA, and a method of separating RNA from DNA, protein, and polysaccharide contaminants. For example, total RNA can be isolated by freezing tissue in

30 liquid nitrogen, grinding the frozen tissue with a mortar and pestle to lyse the cells, extracting the ground tissue with a solution of phenol/chloroform to remove proteins, and separating RNA from the remaining impurities by selective precipitation with lithium chloride (see, for

35 example, Ausubel *et al.* (eds.), *Short Protocols in Molecular Biology, 3rd Edition*, pages 4-1 to 4-6 (John Wiley & Sons 1995) ["Ausubel (1995)"]; Wu *et al.*, *Methods in Gene*

Biotechnology, pages 33-41 (CRC Press, Inc. 1997) ["Wu
(1997)"]).

        Alternatively, total RNA can be isolated from
5   brain or spinal cord tissue as well as heart, skeletal
    muscle, stomach, pancreas, adrenal gland, salivary gland,
    liver, small intestine, bone marrow, thymus, spleen, lymph
    node, thyroid, trachea, testis, ovary or placenta by
    extracting ground tissue with guanidinium isothiocyanate,
10 extracting with organic solvents, and separating RNA from
    contaminants using differential centrifugation (see, for
    example, Chirgwin *et al.*, *Biochemistry 18*:52 (1979);
    Ausubel (1995) at pages 4-1 to 4-6; Wu (1997) at pages 33-
    41). To construct a cDNA library, poly(A)$^+$ RNA must be
15 isolated from a total RNA preparation. Poly(A)$^+$ RNA can be
    isolated from total RNA using the standard technique of
    oligo(dT)-cellulose chromatography (see, for example, Aviv
    and Leder, *Proc. Nat'l Acad. Sci. USA 69*:1408 (1972);
    Ausubel (1995) at pages 4-11 to 4-12). Double-stranded cDNA
20 molecules are synthesized from poly(A)$^+$ RNA using
    techniques well-known to those in the art. (see, for
    example, Wu (1997) at pages 41-46). Moreover,
    commercially available kits can be used to synthesize
    double-stranded cDNA molecules. For example, such kits
25 are available from Life Technologies, Inc. (Gaithersburg,
    MD), CLONTECH Laboratories, Inc. (Palo Alto, CA), Promega
    Corporation (Madison, WI) and STRATAGENE (La Jolla, CA).

        Various cloning vectors are appropriate for the
30 construction of a cDNA library. For example, a cDNA
    library can be prepared in a vector derived from
    bacteriophage, such as a λgt10 vector. See, for example,
    Huynh *et al.*, "Constructing and Screening cDNA Libraries
    in λgt10 and λgt11," in *DNA Cloning: A Practical Approach
35 Vol. I*, Glover (ed.), page 49 (IRL Press, 1985); Wu (1997)
    at pages 47-52. Alternatively, double-stranded cDNA
    molecules can be inserted into a plasmid vector, such as a

pBLUESCRIPT vector (STRATAGENE; La Jolla, CA), a LAMDAGEM-4
(Promega Corp.) or other commercially available vectors.
Suitable cloning vectors also can be obtained from the
American Type Culture Collection (Manassas, VA). To amplify
5   the cloned cDNA molecules, the cDNA library is inserted
into a prokaryotic host, using standard techniques. For
example, a, cDNA library can be introduced into competent
E. coli DH5 cells, which can be obtained, for example, from
Life Technologies, Inc. (Gaithersburg, MD).
10

A human genomic library can be prepared by means
well-known in the art (see, for example, Ausubel (1995) at
pages 5-1 to 5-6; Wu (1997) at pages 307-327). Genomic DNA
can be isolated by lysing tissue with the detergent
15 Sarkosyl, digesting the lysate with proteinase K, clearing
insoluble debris from the lysate by centrifugation,
precipitating nucleic acid from the lysate using
isopropanol, and purifying resuspended DNA on a cesium
chloride density gradient. DNA fragments that are suitable
20 for the production of a genomic library can be obtained by
the random shearing of genomic DNA or by the partial
digestion of genomic DNA with restriction endonucleases.
Genomic DNA fragments can be inserted into a vector, such
as a bacteriophage or cosmid vector, in accordance with
25 conventional techniques, such as the use of restriction
enzyme digestion to provide appropriate termini, the use of
alkaline phosphatase treatment to avoid undesirable joining
of DNA molecules, and ligation with appropriate ligases.
Techniques for such manipulation are well-known in the art
30 (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu
(1997) at pages 307-327).

Nucleic acid molecules that encode a human
Ztgfβ-9 gene can also be obtained using the polymerase
35 chain reaction (PCR) with oligonucleotide primers having
nucleotide sequences that are based upon the nucleotide
sequences of the human *Ztgfβ-9* gene, as described herein.

General methods for screening libraries with PCR are
provided by, for example, Yu et al., "Use of the
Polymerase Chain Reaction to Screen Phage Libraries," in
Methods in Molecular Biology, Vol. 15: PCR Protocols:
5   Current Methods and Applications, White (ed.), pages 211-
215 (Humana Press, Inc. 1993). Moreover, techniques for
using PCR to isolate related genes are described by, for
example, Preston, "Use of Degenerate Oligonucleotide
Primers and the Polymerase Chain Reaction to Clone Gene
10 Family Members," in Methods in Molecular Biology, Vol. 15:
PCR Protocols: Current Methods and Applications, White
(ed.), pages 317-337 (Humana Press, Inc. 1993).
Alternatively, human genomic libraries can be obtained from
commercial sources such as Research Genetics (Huntsville,
15 AL) and the American Type Culture Collection (Manassas,
VA). A library containing cDNA or genomic clones can be
screened with one or more polynucleotide probes based upon
SEQ ID NO:1, using standard methods (see, for example,
Ausubel (1995) at pages 6-1 to 6-11).
20

Anti- Ztgfβ-9 antibodies, produced as described
below, can also be used to isolate DNA sequences that
encode human Ztgfβ-9 genes from cDNA libraries. For
example, the antibodies can be used to screen λgt11
25 expression libraries, or the antibodies can be used for
immunoscreening following hybrid selection and translation
(see, for example, Ausubel (1995) at pages 6-12 to 6-16;
Margolis et al., "Screening λ expression libraries with
antibody and protein probes," in DNA Cloning 2: Expression
30 Systems, 2nd Edition, Glover et al. (eds.), pages 1-14
(Oxford University Press 1995)).

As an alternative, an Ztgfβ-9 gene can be
obtained by synthesizing nucleic acid molecules using
35 mutually priming long oligonucleotides and the nucleotide
sequences described herein (see, for example, Ausubel
(1995) at pages 8-8 to 8-9). Established techniques using

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the polymerase chain reaction provide the ability to
synthesize DNA molecules at least two kilobases in length
(Adang *et al.*, *Plant Molec. Biol.* 21:1131 (1993), Bambot
et al., *PCR Methods and Applications* 2:266 (1993), Dillon
5   *et al.*, "Use of the Polymerase Chain Reaction for the
Rapid Construction of Synthetic Genes," in *Methods in
Molecular Biology, Vol. 15: PCR Protocols: Current Methods
and Applications*, White (ed.), pages 263-268, (Humana
Press, Inc. 1993), and Holowachuk *et al.*, *PCR Methods
10 Appl.* 4:299 (1995)). The sequence of an *Ztgfβ-9* cDNA or
Ztgfβ-9 genomic fragment can be determined using standard
methods. Moreover, the identification of genomic
fragments containing an *Ztgfβ-9* promoter or regulatory
element can be achieved using well-established techniques,
15 such as deletion analysis (see, generally, Ausubel
(1995)).

    Cloning of 5' flanking sequences also
facilitates production of Ztgfβ-9 proteins by "gene
20 activation," following the methods disclosed in U.S.
Patent No. 5,641,670. Briefly, expression of an
endogenous *Ztgfβ-9* gene in a cell is altered by introducing
into the *Ztgfβ-9* locus a DNA construct comprising at least
a targeting sequence, a regulatory sequence, an exon, and
25 an unpaired splice donor site. The targeting sequence is
a *Ztgfβ-9* 5' non-coding sequence that permits homologous
recombination of the construct with the endogenous *Ztgfβ-9*
locus, whereby the sequences within the construct become
operably linked with the endogenous *Ztgfβ-9* coding
30 sequence. In this way, an endogenous *Ztgfβ-9* promoter can
be replaced or supplemented with other regulatory
sequences to provide enhanced, tissue-specific, or
otherwise regulated expression.

35   Additionally, the polynucleotides of the present
invention can be synthesized using a DNA synthesizer.
Currently the method of choice is the phosphoramidite

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method. If chemically synthesized double stranded DNA is
required for an application such as the synthesis of a
gene or a gene fragment, then each complementary strand is
made separately. The production of short genes (60 to 80
5   bp) is technically straightforward and can be accomplished
by synthesizing the complementary strands and then
annealing them. For the production of longer genes (>300
bp), however, special strategies must be invoked, because
the coupling efficiency of each cycle during chemical DNA
10   synthesis is seldom 100%. To overcome this problem,
synthetic genes (double-stranded) are assembled in modular
form from single-stranded fragments that are from 20 to
100 nucleotides in length. In addition to the protein
coding sequence, synthetic genes can be designed with
15   terminal sequences that facilitate insertion into a
restriction endonuclease sites of a cloning vector and
other sequences should also be added that contain signals
for the proper initiation and termination of transcription
and translation. See Glick, Bernard R. and Jack J.
20   Pasternak, *Molecular Biotechnology, Principles &
Applications of Recombinant DNA*, (ASM Press, Washington,
D.C. 1994), Itakura, K. *et al.* Synthesis and use of
synthetic oligonucleotides. *Annu. Rev. Biochem. 53* : 323-
356 (1984), and Climie, S. *et al.* Chemical synthesis of
25   the thymidylate synthase gene. *Proc. Natl. Acad. Sci. USA
87* :633-637 (1990).

        Within preferred embodiments of the invention
the isolated polynucleotides will hybridize to similar
sized regions of the DNA of SEQ ID NO:1, or a sequence
30   complementary thereto, under stringent conditions. In
general, stringent conditions are selected to be about 5°C
lower than the thermal melting point (T_m) for the specific
sequence at a defined ionic strength and pH. The T_m is
35   the temperature (under defined ionic strength and pH) at
which 50% of the target sequence hybridizes to a perfectly
matched probe. Typical stringent conditions are those in

30

which the salt concentration is about 0.02 M or less at pH
7 and the temperature is at least about 60°C. As
previously noted, the isolated polynucleotides of the
present invention include DNA and RNA. Methods for
5   isolating DNA and RNA are well known in the art. Total RNA
can be prepared using guanidine HCl extraction followed by
isolation by centrifugation in a CsCl gradient [Chirgwin
et al., Biochemistry 18:52-94 (1979)]. Poly (A)$^+$ RNA is
prepared from total RNA using the method of Aviv and
10   Leder, Proc. Natl. Acad. Sci. USA 69:1408-1412 (1972).
Complementary DNA (cDNA) is prepared from poly(A)$^+$ RNA
using known methods. Polynucleotides encoding Ztgfβ-9
polypeptides are then identified and isolated by, for
example, hybridization or PCR.
15

        Those skilled in the art will recognize that the
sequences disclosed in SEQ ID NOS:1 and 2 represent a
single allele of the human. There are a number of
20   naturally occurring mature N-terminal variants having the
leader sequence cleaved at differing positions. Allelic
variants of these sequences can be cloned by probing cDNA
or genomic libraries from different individuals according
to standard procedures. The present invention further
25   provides counterpart proteins and polynucleotides from
other species ("species orthologs"). Of particular
interest are Ztgfβ-9 polypeptides from other mammalian
species, including murine, porcine, ovine, bovine, canine,
feline, equine, and other primates. Species orthologs of
30   the human Ztgfβ-9 protein can be cloned using information
and compositions provided by the present invention in
combination with conventional cloning techniques. For
example, a cDNA can be cloned using mRNA obtained from a
tissue or cell type that expresses the gene. Suitable
35   sources of mRNA can be identified by probing Northern
blots with probes designed from the sequences disclosed
herein. A library is then prepared from mRNA of a

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positive tissue or cell line. A protein-encoding cDNA can
then be isolated by a variety of methods, such as by
probing with a complete or partial human cDNA or with one
or more sets of degenerate probes based on the disclosed
sequences. A cDNA can also be cloned using the polymerase
chain reaction, or PCR (Mullis, U.S. Patent No.
4,683,202), using primers designed from the sequences
disclosed herein. Within an additional method, the cDNA
library can be used to transform or transfect host cells,
and expression of the cDNA of interest can be detected
with an antibody to the protein. Similar techniques can
also be applied to the isolation of genomic clones. As
used and claimed the language "an isolated polynucleotide
which encodes a polypeptide, said polynucleotide being
defined by SEQ ID NO: 2" includes all allelic variants and
species orthologs of the polypeptide of SEQ ID NOs:2, 3, 4
and 5.

        Within preferred embodiments of the invention,
isolated nucleic acid molecules that encode human Ztgfβ-9
can hybridize to nucleic acid molecules having the
nucleotide sequence of SEQ ID NO:1, or a sequence
complementary thereto, under "stringent conditions." In
general, stringent conditions are selected to be about 5°C
lower than the thermal melting point (T_m) for the specific
sequence at a defined ionic strength and pH. The T_m is
the temperature (under defined ionic strength and pH) at
which 50% of the target sequence hybridizes to a perfectly
matched probe.

        As an illustration, a nucleic acid molecule
encoding a variant Ztgfβ-9 polypeptide can be hybridized
with a nucleic acid molecule having the nucleotide
sequence of SEQ ID NO:1 (or its complement) at 42°C
overnight in a solution comprising 50% formamide, 5xSSC
(1xSSC: 0.15 M sodium chloride and 15 mM sodium citrate),
50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution

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(100x Denhardt's solution: 2% (w/v) Ficoll 400, 2% (w/v)
polyvinylpyrrolidone, and 2% (w/v) bovine serum albumin),
10% dextran sulfate, and 20 µg/ml denatured, sheared
salmon sperm DNA. One of skill in the art can devise
5   variations of these hybridization conditions. For
example, the hybridization mixture can be incubated at a
higher temperature, such as about 65°C, in a solution that
does not contain formamide. Moreover, premixed
hybridization solutions are available (e.g., EXPRESSHYB
10   Hybridization Solution from CLONTECH Laboratories, Inc.),
and hybridization can be performed according to the
manufacturer's instructions. Following hybridization, the
nucleic acid molecules can be washed to remove non-
hybridized nucleic acid molecules under stringent
15   conditions, or under highly stringent conditions. Typical
stringent washing conditions include washing in a solution
of 0.5x - 2x SSC with 0.1% sodium dodecyl sulfate (SDS) at
55 - 65°C. That is, nucleic acid molecules encoding a
variant Ztgfβ-9 polypeptide hybridize with a nucleic acid
20   molecule having the nucleotide sequence of SEQ ID NO:1 (or
its complement) under stringent washing conditions, in
which the wash stringency is equivalent to 0.5x - 2x SSC
with 0.1% SDS at 55 - 65°C, including 0.5x SSC with 0.1%
SDS at 55°C, or 2xSSC with 0.1% SDS at 65°C. One of skill
25   in the art can readily devise equivalent conditions, for
example, by substituting SSPE for SSC in the wash
solution.

Typical highly stringent washing conditions
30   include washing in a solution of 0.1x - 0.2x SSC with 0.1%
sodium dodecyl sulfate (SDS) at 50 - 65°C. In other
words, nucleic acid molecules encoding a variant Ztgfβ-9
polypeptide hybridize with a nucleic acid molecule having
the nucleotide sequence of SEQ ID NO:1 (or its complement)
35   under highly stringent washing conditions, in which the
wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1%

SDS at 50 - 65°C, including 0.1x SSC with 0.1% SDS at
50°C, or 0.2xSSC with 0.1% SDS at 65°C.

     The present invention also provides isolated
5   Ztgfβ-9 polypeptides that have a substantially similar
    sequence identity to the polypeptides of SEQ ID NOs:2,3,
    4, 5, 9, 12, 17, 18 or their orthologs. The term
    "substantially similar sequence identity" is used herein
    to denote polypeptides having at least 70%, at least 80%,
10 at least 90%, at least 95% or greater than 95% and 99%
    sequence identity to the sequences shown in SEQ ID NOs:2,
    3, 4, 5, 9, 12, 17, 18 or their orthologs.

     The present invention also contemplates Ztgfβ-9
15 variant nucleic acid molecules that can be identified
    using two criteria: a determination of the similarity
    between the encoded polypeptide with the amino acid
    sequence of SEQ ID NOs:2, 3, 4, 5, 9, 12, 17 or 18, and a
    hybridization assay, as described above. Such Ztgfβ-9
20 variants include nucleic acid molecules (1) that hybridize
    with a nucleic acid molecule having the nucleotide
    sequence of SEQ ID NO:1, SEQ ID NO: 9 or SEQ ID NO:16 (or
    their complement) under stringent washing conditions, in
    which the wash stringency is equivalent to 0.5x - 2x SSC
25 with 0.1% SDS at 55 - 65°C, and (2) that encode a
    polypeptide having at least 70%, at least 80%, at least
    90%, at least 95% or greater than 95% sequence identity to
    the amino acid sequence of SEQ ID NOs:2, 3, 4, 5, 9, 12,
    17 or 18. Alternatively, Ztgfβ-9 variants can be
30 characterized as nucleic acid molecules (1) that hybridize
    with a nucleic acid molecule having the nucleotide
    sequence of SEQ ID NO:1 (or its complement) under highly
    stringent washing conditions, in which the wash stringency
    is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 -
35 65°C, and (2) that encode a polypeptide having at least
    70%, at least 80%, at least 90%, at least 95% or greater

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than 95% or 99% sequence identity to the amino acid
sequence of SEQ ID NOs:2, 3, 4, 5, 9, 12, 17 or 18.

5   The present invention also contemplates human
Ztgfβ-9 variant nucleic acid molecules identified by at
least one of hybridization analysis and sequence identity
determination, with reference to SEQ ID NO:2.   The present
invention further includes murine *Ztgfβ-9* variant nucleic
acid molecules identified by at least one of hybridization
10   analysis and sequence identity determination, with
reference to SEQ ID NOs:8 and 9.   For example, using the
approach discussed above, murine Ztgfβ-9 variant nucleic
acid molecules can be identified using at least one of
three criteria: (1) hybridization with a nucleic acid
15   molecule having the nucleotide sequence of SEQ ID NO:8(or
its complement) under stringent washing conditions, in
which the wash stringency is equivalent to 0.5x - 2x SSC
with 0.1% SDS at 55 - 65°C, (2) hybridization with a
nucleic acid molecule having the nucleotide sequence of
20   SEQ ID NO:8 (or its complement) under highly stringent
washing conditions , in which the wash stringency is
equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50 - 65°C,
and (3) an amino acid percent identity that is at least
70%, at least 80%, at least 90%, at least 95% or greater
25   than 95% sequence identity to the amino acid sequence of
SEQ ID NO:12.

        Percent sequence identity is determined by
conventional methods.   See, for example, Altschul *et al.*,
30   *Bull. Math. Bio. 48*:603 (1986), and Henikoff and Henikoff,
Proc. Natl. Acad. Sci. USA 89:10915 (1992).   Briefly, two
amino acid sequences are aligned to optimize the alignment
scores using a gap opening penalty of 10, a gap extension
penalty of 1, and the "BLOSUM 62" scoring matrix of
35   Henikoff and Henikoff (*ibid.*) as shown in Table 1 (amino
acids are indicated by the standard one-letter codes).
The percent identity is then calculated as: ([Total number

of identical matches]/ [length of the longer sequence plus
the number of gaps introduced into the longer sequence in
order to align the two sequences]) (100).

Table 1

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

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Those skilled in the art appreciate that there
are many established algorithms available to align two
amino acid sequences. The "FASTA" similarity search
algorithm of Pearson and Lipman is a suitable protein
5   alignment method for examining the level of identity
shared by an amino acid sequence disclosed herein and the
amino acid sequence of a putative Ztgfβ-9 variant. The
FASTA algorithm is described by Pearson and Lipman, *Proc.
Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth.*
10   *Enzymol. 183*:63 (1990). Briefly, FASTA first
characterizes sequence similarity by identifying regions
shared by the query sequence (*e.g.*, SEQ ID NO:2) and a
test sequence that have either the highest density of
identities (if the ktup variable is 1) or pairs of
15   identities (if ktup=2), without considering conservative
amino acid substitutions, insertions, or deletions. The
ten regions with the highest density of identities are
then re-scored by comparing the similarity of all paired
amino acids using an amino acid substitution matrix, and
20   the ends of the regions are "trimmed" to include only
those residues that contribute to the highest score. If
there are several regions with scores greater than the
"cutoff" value (calculated by a predetermined formula
based upon the length of the sequence and the ktup value),
25   then the trimmed initial regions are examined to determine
whether the regions can be joined to form an approximate
alignment with gaps. Finally, the highest scoring regions
of the two amino acid sequences are aligned using a
modification of the Needleman-Wunsch-Sellers algorithm
30   (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970);
Sellers, *SIAM J. Appl. Math. 26*:787 (1974)), which allows
for amino acid insertions and deletions. Illustrative
parameters for FASTA analysis are: ktup=1, gap opening
penalty=10, gap extension penalty=1, and substitution
35   matrix=BLOSUM62. These parameters can be introduced into
a FASTA program by modifying the scoring matrix file

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("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol.* *183*:63 (1990).

5   FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as described above.

10

The present invention includes nucleic acid molecules that encode a polypeptide having a conservative amino acid change, compared with the amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO:5,

15   SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:17 or SEQ ID NO:18. That is, variants can be obtained that contain one or more amino acid substitutions of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO: 4 SEQ ID NO:5 SEQ ID NO:9 or SEQ ID NO:12, in which an alkyl amino acid is substituted for an alkyl amino acid

20   in a Ztgfβ-9 amino acid sequence, an aromatic amino acid is substituted for an aromatic amino acid in an Ztgfβ-9 amino acid sequence, a sulfur-containing amino acid is substituted for a sulfur-containing amino acid in an Ztgfβ-9 amino acid sequence, a hydroxy-containing amino

25   acid is substituted for a hydroxy-containing amino acid in a Ztgfβ-9 amino acid sequence, an acidic amino acid is substituted for an acidic amino acid in a Ztgfβ-9 amino acid sequence, a basic amino acid is substituted for a basic amino acid in a Ztgfβ-9 amino acid sequence, or a

30   dibasic monocarboxylic amino acid is substituted for a dibasic monocarboxylic amino acid in an Ztgfβ-9 amino acid sequence.

Among the common amino acids, for example, a

35   "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine,

and isoleucine, (2) phenylalanine, tyrosine, and
tryptophan, (3) serine and threonine, (4) aspartate and
glutamate, (5) glutamine and asparagine, and (6) lysine,
arginine and histidine. For example, variant Ztgfβ-9
polypeptides that have an amino acid sequence that differs
from either SEQ ID NOs:2, 3, 4, 5 or 12 can be obtained by
substituting a threonine residue for Ser, by substituting
a valine residue for Ile, by substituting an aspartate
residue for Glu, or by substituting a valine residue for
Ile. Additional variants can be obtained by producing
polypeptides having two or more of these amino acid
substitutions.

Variants of either the human or the murine
Ztgfβ-9 can be devised by aligning the amino acid
sequences of SEQ ID NO:3 and SEQ ID NO:12, and by noting
any differences in the corresponding amino acid residues.

The BLOSUM62 table is an amino acid substitution
matrix derived from about 2,000 local multiple alignments
of protein sequence segments, representing highly
conserved regions of more than 500 groups of related
proteins (Henikoff and Henikoff, *Proc. Nat'l Acad. Sci.
USA 89*:10915 (1992)). Accordingly, the BLOSUM62
substitution frequencies can be used to define
conservative amino acid substitutions that may be
introduced into the amino acid sequences of the present
invention. Although it is possible to design amino acid
substitutions based solely upon chemical properties (as
discussed above), the language "conservative amino acid
substitution" preferably refers to a substitution
represented by a BLOSUM62 value of greater than -1. For
example, an amino acid substitution is conservative if the
substitution is characterized by a BLOSUM62 value of 0,
1, 2, or 3. According to this system, preferred
conservative amino acid substitutions are characterized by
a BLOSUM62 value of at least 1 (*e.g.*, 1, 2 or 3), while

more preferred conservative amino acid substitutions are
characterized by a BLOSUM62 value of at least 2 (e.g., 2
or 3).

5   Particular variants of human or murine Ztgfβ-9
are characterized by having at least 70%, at least 80%, at
least 90%, at least 95% or 99% or greater sequence
identity to the corresponding human (i.e., SEQ ID NOs: 2,
3, 4, 5 or 17) or murine (i.e., SEQ ID NOs:9 or 12) amino
10   acid sequences, wherein the variation in amino acid
sequence is due to one or more conservative amino acid
substitutions.

          Conservative amino acid changes in a Ztgfβ-9
15   gene can be introduced by substituting nucleotides for the
nucleotides recited in any one of SEQ ID NOs:1 or 9. Such
"conservative amino acid" variants can be obtained, for
example, by oligonucleotide-directed mutagenesis, linker-
scanning mutagenesis, mutagenesis using the polymerase
20   chain reaction, and the like (see Ausubel (1995) at pages
8-10 to 8-22; and McPherson (ed.), Directed Mutagenesis: A
Practical Approach (IRL Press 1991)). The ability of such
variants to promote anti-viral or anti-proliferative
activity can be determined using a standard method, such
25   as the assay described herein. Alternatively, a variant
Ztgfβ-9 polypeptide can be identified by the ability to
specifically bind anti- Ztgfβ-9 antibodies.

          The proteins of the present invention can also
30   comprise non-naturally occurring amino acid residues.
Non-naturally occurring amino acids include, without
limitation, trans-3-methylproline, 2,4-methanoproline,
cis-4-hydroxyproline, trans-4-hydroxyproline, N-
methylglycine, allo-threonine, methylthreonine,
35   hydroxyethylcysteine, hydroxyethylhomocysteine,
nitroglutamine, homoglutamine, pipecolic acid,
thiazolidine carboxylic acid, dehydroproline, 3- and 4-

methylproline, 3,3-dimethylproline, *tert*-leucine,
norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-
azaphenylalanine, and 4-fluorophenylalanine. Several
methods are known in the art for incorporating non-
5   naturally occurring amino acid residues into proteins.
For example, an *in vitro* system can be employed wherein
nonsense mutations are suppressed using chemically
aminoacylated suppressor tRNAs. Methods for synthesizing
amino acids and aminoacylating tRNA are known in the art.
10   Transcription and translation of plasmids containing
nonsense mutations is typically carried out in a cell-free
system comprising an *E. coli* S30 extract and commercially
available enzymes and other reagents. Proteins are
purified by chromatography. See, for example, Robertson
15   *et al.*, *J. Am. Chem. Soc. 113*:2722 (1991), Ellman *et al.*,
Methods Enzymol. 202:301 (1991), Chung *et al.*, *Science
259*:806 (1993), and Chung *et al.*, *Proc. Nat'l Acad. Sci.
USA 90*:10145 (1993).

20   In a second method, translation is carried out
in *Xenopus* oocytes by microinjection of mutated mRNA and
chemically aminoacylated suppressor tRNAs (Turcatti *et
al.*, *J. Biol. Chem. 271*:19991 (1996)). Within a third
method, *E. coli* cells are cultured in the absence of a
25   natural amino acid that is to be replaced (*e.g.*,
phenylalanine) and in the presence of the desired non-
naturally occurring amino acid(s) (*e.g.*, 2-
azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine,
or 4-fluorophenylalanine). The non-naturally occurring
30   amino acid is incorporated into the protein in place of
its natural counterpart. See, Koide *et al.*, *Biochem.
33*:7470 (1994). Naturally occurring amino acid residues
can be converted to non-naturally occurring species by *in
vitro* chemical modification. Chemical modification can be
35   combined with site-directed mutagenesis to further expand
the range of substitutions (Wynn and Richards, *Protein
Sci. 2*:395 (1993)).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for Ztgfβ-9 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081 (1989), Bass *et al.*, *Proc. Nat'l Acad. Sci. USA 88*:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in *Proteins: Analysis and Design*, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, *J. Biol. Chem. 271*:4699 (1996).

Table 2

Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan

                        tyrosine
        Small: glycine   .
                        alanine
                        serine
5   threonine
                        methionine

        Essential amino acids in the polypeptides of the
present invention can be identified according to
10   procedures known in the art, such as site-directed
mutagenesis or alanine-scanning mutagenesis [Cunningham
and Wells, *Science 244*: 1081-1085 (1989); Bass *et al.*,
Proc. Natl. Acad. Sci. USA 88:4498-4502 (1991)].   In the
latter technique, single alanine mutations are introduced
15   at every residue in the molecule, and the resultant mutant
molecules are tested for biological activity (e.g., ligand
binding and signal transduction) to identify amino acid
residues that are critical to the activity of the
molecule.   Sites of ligand-protein interaction can also be
20   determined by analysis of crystal structure as determined
by such techniques as nuclear magnetic resonance,
crystallography or photoaffinity labeling.   See, for
example, de Vos *et al.*, *Science 255*:306-312 (1992); Smith
et al., *J. Mol. Biol. 224*:899-904, 1992; *Wlodaver et al.*,
25   *FEBS Lett. 309*:59-64 (1992).   The identities of essential
amino acids can also be inferred from analysis of
homologies with related proteins.

        Multiple amino acid substitutions can be made
30   and tested using known methods of mutagenesis and
screening, such as those disclosed by Reidhaar-Olson and
Sauer, *Science 241*:53-57 (1988) or Bowie and Sauer, *Proc.*
Natl. Acad. Sci. USA 86:2152-2156 (1989).   Briefly, these
authors disclose methods for simultaneously randomizing
35   two or more positions in a polypeptide, selecting for
functional polypeptide, and then sequencing the
mutagenized polypeptides to determine the spectrum of

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allowable substitutions at each position. Other methods
that can be used include phage display, *e.g.*, Lowman *et
al.*, *Biochem. 30*:10832-10837 (1991); Ladner *et al.*, U.S.
Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204)
5   and region-directed mutagenesis, Derbyshire *et al.*, *Gene
46*:145 (1986); Ner *et al.*, *DNA 7*:127 (1988).

        Mutagenesis methods as disclosed above can be
combined with high-throughput screening methods to detect
10   activity of cloned, mutagenized proteins in host cells.
Preferred assays in this regard include cell proliferation
assays and biosensor-based ligand-binding assays, which
are described below. Mutagenized DNA molecules that encode
active proteins or portions thereof (e.g., ligand-binding
15   fragments) can be recovered from the host cells and
rapidly sequenced using modern equipment. These methods
allow the rapid determination of the importance of
individual amino acid residues in a polypeptide of
interest, and can be applied to polypeptides of unknown
20   structure.

        Using the methods discussed above, one of
ordinary skill in the art can prepare a variety of
polypeptides that are substantially identical to SEQ ID
25   NOs: 2, 3, 4, 5, 9, 12, 17 or 18 or allelic variants
thereof and retain the properties of the wild-type
protein. As expressed and claimed herein the language, "a
polypeptide as defined by SEQ ID NOs: 2, 3, 4, 5, 9, 12,
17 or 18" includes all allelic variants and species
30   orthologs of the polypeptide.

        Another embodiment of the present invention
provides for a peptide or polypeptide comprising an
epitope-bearing portion of a polypeptide of the invention.
35   The epitope of the this polypeptide portion is an
immunogenic or antigenic epitope of a polypeptide of the
invention. A region of a protein to which an antibody can

45

bind is defined as an "antigenic epitope". See for
instance, Geysen, H.M. *et al., Proc. Natl. Acad Sci. USA
81:3998-4002* (1984).

5   As to the selection of peptides or polypeptides
bearing an antigenic epitope (*i.e.*, that contain a region
of a protein molecule to which an antibody can bind), it
is well known in the art that relatively short synthetic
peptides that mimic part of a protein sequence are
10   routinely capable of eliciting an antiserum that reacts
with the partially mimicked protein. See Sutcliffe, J.G.
et al. Science 219:660-666 (1983). Peptides capable of
eliciting protein-reactive sera are frequently represented
in the primary sequence of a protein, can be characterized
15   by a set of simple chemical rules, and are confined
neither to immunodominant regions of intact proteins
(*i.e.*, immunogenic epitopes) nor to the amino or carboxyl
termini. Peptides that are extremely hydrophobic and those
of six or fewer residues generally are ineffective at
20   inducing antibodies that bind to the mimicked protein;
longer soluble peptides, especially those containing
proline residues, usually are effective.

          Antigenic epitope-bearing peptides and
25   polypeptides of the invention are therefore useful to
raise antibodies, including monoclonal antibodies, that
bind specifically to a polypeptide of the invention.
Antigenic epitope-bearing peptides and polypeptides of the
present invention contain a sequence of at least nine,
30   preferably between 15 to about 30 amino acids contained
within the amino acid sequence of a polypeptide of the
invention. However, peptides or polypeptides comprising a
larger portion of an amino acid sequence of the invention,
containing from 30 to 50 amino acids, or any length up to
35   and including the entire amino acid sequence of a
polypeptide of the invention, also are useful for inducing
antibodies that react with the protein. Preferably, the

amino acid sequence of the epitope-bearing peptide is
selected to provide substantial solubility in aqueous
solvents (*i.e.*, the sequence includes relatively
hydrophilic residues and hydrophobic residues are
5   preferably avoided); and sequences containing proline
residues are particularly preferred. All of the
polypeptides shown in the sequence listing contain
antigenic epitopes to be used according to the present
invention.
10

          Polynucleotides, generally a cDNA sequence, of
the present invention encode the above-described
polypeptides. A cDNA sequence which encodes a polypeptide
of the present invention is comprised of a series of
15   codons, each amino acid residue of the polypeptide being
encoded by a codon and each codon being comprised of three
nucleotides. The amino acid residues are encoded by their
respective codons as follows.

20   Alanine (Ala) is encoded by GCA, GCC, GCG or
GCT;
              Cysteine (Cys) is encoded by TGC or TGT;
              Aspartic acid (Asp) is encoded by GAC or GAT;
              Glutamic acid (Glu) is encoded by GAA or GAG;
25   Phenylalanine (Phe) is encoded by TTC or TTT;
              Glycine (Gly) is encoded by GGA, GGC, GGG or
GGT;
              Histidine (His) is encoded by CAC or CAT;
              Isoleucine (Ile) is encoded by ATA, ATC or ATT;
30   Lysine (Lys) is encoded by AAA, or AAG;
              Leucine (Leu) is encoded by TTA, TTG, CTA, CTC,
CTG or CTT;
              Methionine (Met) is encoded by ATG;
              Asparagine (Asn) is encoded by AAC or AAT;
35   Proline (Pro) is encoded by CCA, CCC, CCG or
CCT;
              Glutamine (Gln) is encoded by CAA or CAG;

Arginine (Arg) is encoded by AGA, AGG, CGA, CGC, CGG or CGT;

Serine (Ser) is encoded by AGC, AGT, TCA, TCC, TCG or TCT;

5   Threonine (Thr) is encoded by ACA, ACC, ACG or ACT;

Valine (Val) is encoded by GTA, GTC, GTG or GTT;

Tryptophan (Trp) is encoded by TGG; and

Tyrosine (Tyr) is encoded by TAC or TAT.

10

It is to be recognized that according to the present invention, when a cDNA is claimed as described above, it is understood that what is claimed are both the sense strand, the anti-sense strand, and the DNA as 15 double-stranded having both the sense and anti-sense strand annealed together by their respective hydrogen bonds. Also claimed is the messenger RNA (mRNA) which encodes the polypeptides of the present invention, and which mRNA is encoded by the above-described cDNA. A 20 messenger RNA (mRNA) will encode a polypeptide using the same codons as those defined above, with the exception that each thymine nucleotide (T) is replaced by a uracil nucleotide (U).

25   The protein polypeptides of the present invention, including full-length proteins, protein fragments (e.g. receptor-binding fragments), and fusion polypeptides can be produced in genetically engineered host cells according to conventional techniques. Suitable 30 host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. 35 Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook *et al.*, *Molecular Cloning: A*

Laboratory Manual, (2nd ed.) (Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, NY, 1989).

        In general, a DNA sequence encoding a Ztgfβ-9
5 polypeptide is operably linked to other genetic elements
required for its expression, generally including a
transcription promoter and terminator, within an
expression vector. The vector will also commonly contain
one or more selectable markers and one or more origins of
10 replication, although those skilled in the art will
recognize that within certain systems selectable markers
may be provided on separate vectors, and replication of
the exogenous DNA may be provided by integration into the
host cell genome. Selection of promoters, terminators,
15 selectable markers, vectors and other elements is a matter
of routine design within the level of ordinary skill in
the art. Many such elements are described in the
literature and are available through commercial suppliers.

20   To direct a Ztgfβ-9 polypeptide into the
secretory pathway of a host cell, a secretory signal
sequence (also known as a leader sequence, prepro sequence
or pre sequence) is provided in the expression vector.
The secretory signal sequence may be that of the protein,
25 or may be derived from another secreted protein [*e.g.*, the
tissue plasminogen activator (t-PA)] leader sequence or
synthesized *de novo*. The secretory signal sequence is
joined to the *Ztgfβ*-9 DNA sequence in the correct reading
frame. Secretory signal sequences are commonly positioned
30 5' to the DNA sequence encoding the polypeptide of
interest, although certain signal sequences may be
positioned elsewhere in the DNA sequence of interest (see,
e.g., Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et
al.*, U.S. Patent No. 5,143,830).
35

        Cultured mammalian cells are preferred hosts
within the present invention. Methods for introducing

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exogenous DNA into mammalian host cells include calcium
phosphate-mediated transfection, Wigler et al., *Cell*
14:725 (1978); Corsaro and Pearson, *Somatic Cell Genetics*
7:603 (1981): Graham and Van der Eb, *Virology 52*:456
(1973), electroporation, Neumann et al., *EMBO J. 1*:841-845
(1982), DEAE-dextran mediated transfection, Ausubel et
al., eds., *Current Protocols in Molecular Biology* (John
Wiley and Sons, Inc., NY, 1987), and liposome-mediated
transfection (Hawley-Nelson et al., *Focus 15*:73 (1993);
Ciccarone et al., *Focus 15*:80 (1993). The production of
recombinant polypeptides in cultured mammalian cells is
disclosed, for example, by Levinson et al., U.S. Patent
No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950;
Palmiter et al., U.S. Patent No. 4,579,821; and Ringold,
U.S. Patent No. 4,656,134. Suitable cultured mammalian
cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC
No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No.
CRL 10314), 293 [ATCC No. CRL 1573; Graham et al., *J. Gen.*
Virol. 36:59-72 (1977)] and Chinese hamster ovary (e.g.
CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable
cell lines are known in the art and available from public
depositories such as the American Type Culture Collection,
Rockville, Maryland. In general, strong transcription
promoters are preferred, such as promoters from SV-40 or
cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288.
Other suitable promoters include those from
metallothionein genes (U.S. Patent Nos. 4,579,821 and
4,601,978) and the adenovirus major late promoter.

          Other higher eukaryotic cells can also be used
as hosts, including plant cells, insect cells and avian
cells. The use of *Agrobacterium rhizogenes* as a vector
for expressing genes in plant cells has been reviewed by
Sinkar et al., *J. Biosci. (Bangalore) 11*:47 (1987).
Transformation of insect cells and production of foreign
polypeptides therein is disclosed by Guarino et al., U.S.
Patent No. 5,162,222 and WIPO publication WO 94/06463.

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Insect cells can be infected with recombinant baculovirus, commonly derived from Autographa californica nuclear polyhedrosis virus (AcNPV). DNA encoding the Ztgfβ-9 polypeptide is inserted into the baculoviral genome in

5   place of the AcNPV polyhedrin gene coding sequence by one of two methods. The first is the traditional method of homologous DNA recombination between wild-type AcNPV and a transfer vector containing the Ztgfβ-9 cDNA flanked by AcNPV sequences. Suitable insect cells, e.g. SF9 cells,

10   are infected with wild-type AcNPV and transfected with a transfer vector comprising a Ztgfβ-9 polynucleotide operably linked to an AcNPV polyhedrin gene promoter, terminator, and flanking sequences. See, King, L.A. and Possee, R.D., The Baculovirus Expression System: A

15   Laboratory Guide, (Chapman & Hall, London); O'Reilly, D.R. et al., Baculovirus Expression Vectors: A Laboratory Manual (Oxford University Press, New York, New York, 1994); and, Richardson, C. D., Ed., Baculovirus Expression Protocols. Methods in Molecular Biology, (Humana Press,

20   Totowa, NJ 1995). Natural recombination within an insect cell will result in a recombinant baculovirus which contains Ztgfβ-9 driven by the polyhedrin promoter. Recombinant viral stocks are made by methods commonly used in the art.

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The second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow, V.A, et al., J Virol 67:4566 (1993). This system is sold in the Bac-to-Bac kit (Life Technologies,

30   Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Ztgfβ-9 polypeptide into a baculovirus genome maintained in E. coli as a large plasmid called a "bacmid." The pFastBac1™ transfer vector

35   utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case Ztgfβ-9.

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However, pFastBacl™ can be modified to a considerable
degree. The polyhedrin promoter can be removed and
substituted with the baculovirus basic protein promoter
(also known as P*cor*, p6.9 or MP promoter) which is

5   expressed earlier in the baculovirus infection, and has
been shown to be advantageous for expressing secreted
proteins. See, Hill-Perkins, M.S. and Possee, R.D., *J Gen
Virol 71*:971 (1990); Bonning, B.C. *et al.*, *J Gen Virol
75*:1551 (1994); and, Chazenbalk, G.D., and Rapoport, B., *J

10   Biol Chem 270*:1543 (1995). In such transfer vector
constructs, a short or long version of the basic protein
promoter can be used. Moreover, transfer vectors can be
constructed which replace the native Ztgfβ-9 secretory
signal sequences with secretory signal sequences derived

15   from insect proteins. For example, a secretory signal
sequence from Ecdysteroid Glucosyltransferase (EGT), honey
bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus
gp67 (PharMingen, San Diego, CA) can be used in constructs
to replace the native Ztgfβ-9 secretory signal sequence.

20   In addition, transfer vectors can include an in-frame
fusion with DNA encoding an epitope tag at the C- or N-
terminus of the expressed Ztgfβ-9 polypeptide, for
example, a Glu-Glu epitope tag, Grussenmeyer, T. *et al.*,
Proc Natl Acad Sci. 82:7952 (1985). Using a technique

25   known in the art, a transfer vector containing *Ztgfβ-9* is
transformed into *E. coli,* and screened for bacmids which
contain an interrupted lacZ gene indicative of recombinant
baculovirus. The bacmid DNA containing the recombinant
baculovirus genome is isolated, using common techniques,

30   and used to transfect *Spodoptera frugiperda* cells, *e.g.*
Sf9 cells. Recombinant virus that expresses Ztgfβ-9 is
subsequently produced. Recombinant viral stocks are made
by methods commonly used the art.

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The recombinant virus is used to infect host cells, typically a cell line derived from the fall army worm, *Spodoptera frugiperda*. See, in general, Glick and

5   Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA*, ASM Press, Washington, D.C. (1994). Another suitable cell line is the High FiveO™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent #5,300,435). Commercially available serum-

10   free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cellO405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. The cells are

15   grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. The recombinant virus-infected cells typically produce the

20   recombinant Ztgfβ-9 polypeptide at 12-72 hours post-infection and secrete it with varying efficiency into the medium. The culture is usually harvested 48 hours post-infection. Centrifugation is used to separate the cells from the medium (supernatant). The supernatant containing

25   the z*** polypeptide is filtered through micropore filters, usually 0.45 μm pore size. Procedures used are generally described in available laboratory manuals (King, L. A. and Possee, R.D., *ibid.*; O'Reilly, D.R. *et al.*, *ibid.*; Richardson, C. D., *ibid.*). Subsequent purification

30   of the Ztgfβ-9 polypeptide from the supernatant can be achieved using methods described herein.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been

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inserted. Such cells are commonly referred to as
"transfectants". Cells that have been cultured in the
presence of the selective agent and are able to pass the
gene of interest to their progeny are referred to as
5   "stable transfectants." A preferred selectable marker is
a gene encoding resistance to the antibiotic neomycin.
Selection is carried out in the presence of a neomycin-
type drug, such as G-418 or the like. Selection systems
may also be used to increase the expression level of the
10 gene of interest, a process referred to as
"amplification." Amplification is carried out by
culturing transfectants in the presence of a low level of
the selective agent and then increasing the amount of
selective agent to select for cells that produce high
15 levels of the products of the introduced genes. A
preferred amplifiable selectable marker is dihydrofolate
reductase, which confers resistance to methotrexate.
Other drug resistance genes (e.g. hygromycin resistance,
multi-drug resistance, and puromycin acetyltransferase)
20 can also be used.

        Other higher eukaryotic cells can also be used
as hosts, including insect cells, plant cells and avian
cells. Transformation of insect cells and production of
25 foreign polypeptides therein is disclosed by Guarino et
al., U.S. Patent No. 5,162,222; Bang et al., U.S. Patent
No. 4,775,624; and WIPO publication WO 94/06463. The use
of Agrobacterium rhizogenes as a vector for expressing
genes in plant cells has been reviewed by Sinkar et al.,
30 J. Biosci. (Bangalore) 11:47-58 (1987).

        Fungal cells, including yeast cells, and
particularly cells of the genus Saccharomyces, can also be
used within the present invention, such as for producing
35 protein fragments or polypeptide fusions. Methods for
transforming yeast cells with exogenous DNA and producing
recombinant polypeptides therefrom are disclosed by, for

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example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki *et al.*, U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch *et al.*, U.S. Patent No. 5,037,743; and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed

5   cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the *POT1* vector system disclosed by Kawasaki *et al.*, U.S.

10 Patent No. 4,931,373, which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, *e.g.*, Kawasaki, U.S. Patent No. 4,599,311; Kingsman *et al.*, U.S. Patent No.

15 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*,

20 *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al.*, *J. Gen. Microbiol.* *132*:3459-3465 (1986) and Cregg, U.S. Patent No. 4,882,279.

25 *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming Neurospora are disclosed by Lambowitz, U.S.

30 Patent No. 4,486,533.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention.

35 Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art, see, *e.g.*, Sambrook *et al.*, *ibid.*). When expressing

a Ztgfβ-9 polypeptide in bacteria such as *E. coli*, the
polypeptide may be retained in the cytoplasm, typically as
insoluble granules, or may be directed to the periplasmic
space by a bacterial secretion sequence. In the former
5   case, the cells are lysed, and the granules are recovered
and denatured using, for example, guanidine isothiocyanate
or urea. The denatured polypeptide can then be refolded
and dimerized by diluting the denaturant, such as by
dialysis against a solution of urea and a combination of
10 reduced and oxidized glutathione, followed by dialysis
against a buffered saline solution. In the latter case,
the polypeptide can be recovered from the periplasmic
space in a soluble and functional form by disrupting the
cells (by, for example, sonication or osmotic shock) to
15 release the contents of the periplasmic space and
recovering the protein, thereby obviating the need for
denaturation and refolding.

Transformed or transfected host cells are
20 cultured according to conventional procedures in a culture
medium containing nutrients and other components required
for the growth of the chosen host cells. A variety of
suitable media, including defined media and complex media,
are known in the art and generally include a carbon
25 source, a nitrogen source, essential amino acids, vitamins
and minerals. Media may also contain such components as
growth factors or serum, as required. The growth medium
will generally select for cells containing the exogenously
added DNA by, for example, drug selection or deficiency in
30 an essential nutrient which is complemented by the
selectable marker carried on the expression vector or co-
transfected into the host cell.

Within one aspect of the present invention, a
35 novel protein is produced by a cultured cell, and the cell
or the protein is used to screen for a receptor or
receptors for the protein, including the natural receptor,

as well as interacting proteins such as dimerization
partners, agonists and antagonists of the natural ligand.

PROTEIN ISOLATION:

Expressed recombinant polypeptides (or chimeric
polypeptides) can be purified using fractionation and/or
conventional purification methods and media. Ammonium
sulfate precipitation and acid or chaotrope extraction may
be used for fractionation of samples. Exemplary
purification steps may include hydroxyapatite, size
exclusion, FPLC and reverse-phase high performance liquid
chromatography. Suitable anion exchange media include
derivatized dextrans, agarose, cellulose, polyacrylamide,
specialty silicas, and the like. PEI, DEAE, QAE and Q
derivatives are preferred, with DEAE Fast-Flow Sepharose
(Pharmacia, Piscataway, NJ) being particularly preferred.
Exemplary chromatographic media include those media
derivatized with phenyl, butyl, or octyl groups, such as
Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso
Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia)
and the like; or polyacrylic resins, such as Amberchrom CG
71 (Toso Haas) and the like. Suitable solid supports
include glass beads, silica-based resins, cellulosic
resins, agarose beads, cross-linked agarose beads,
polystyrene beads, cross-linked polyacrylamide resins and
the like that are insoluble under the conditions in which
they are to be used. These supports may be modified with
reactive groups that allow attachment of proteins by amino
groups, carboxyl groups, sulfhydryl groups, hydroxyl
groups and/or carbohydrate moieties. Examples of coupling
chemistries include cyanogen bromide activation, N-
hydroxysuccinimide activation, epoxide activation,
sulfhydryl activation, hydrazide activation, and carboxyl
and amino derivatives for carbodiimide coupling
chemistries. These and other solid media are well known
and widely used in the art, and are available from

commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988).

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The polypeptides of the present invention can be isolated by exploitation of their *properties*. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins. Briefly, a gel is first charged with divalent metal ions to form a chelate [E. Sulkowski, *Trends in Biochem.* 3:1-7 (1985)]. Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography [*Methods in Enzymol.*, Vol. *182*:529-39, "Guide to Protein Purification", M. Deutscher, (ed.), (Acad. Press, San Diego, 1990). Alternatively, a fusion of the polypeptide of interest and an affinity tag (e.g., polyhistidine, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification. Furthermore, to facilitate purification of the secreted polypeptide, an amino or carboxyl-terminal extension, such as a poly-histidine tag, substance P, FLAG® peptide [Hopp *et al.*, *Bio/Technology* 6:1204-1210 (1988); available from Eastman Kodak Co., New Haven, CT), a Glu-Glu affinity tag [Grussenmeyer *et al.*, *Proc. Natl. Acad. Sci. USA* 82:7952-4 (1985)], or another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to Ztgfβ to aid in purification.

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Mice engineered to express the Ztgfβ-9 gene,
referred to as "transgenic mice" and mice that exhibit a
complete absence of Ztgfβ-9 gene function, referred to as
"knockout mice", may also be generated (Snouwaert *et al.,*
5   *Science,* 257:1083 (1992); Lowell, *et al., Nature,* 366:740-
742 (1993); Capecchi, M.R., *Science,* 244:1288-1292,
(1989); Palmiter, R.D. *et al., Annu. Rev. Genet.,* 20:465-
499, (1986). For example, transgenic mice that over-
express Ztgfβ-9 either ubiquitously or under a tissue-
10 specific or tissue-restricted promoter can be used to ask
whether overexpression causes a phenotype. For example,
overexpression of a wild-type Ztgfβ-9 polypeptide,
polypeptide fragment or a mutant thereof may alter normal
cellular processes resulting in a phenotype that
15 identifies a tissue in which Ztgfβ-9 expression is
functionally relevant and may indicate a therapeutic
target for the Ztgfβ-9 protein, gene, its agonists or
antagonists. Moreover, such over-expression may result in
a phenotype that shows similarity with human diseases.
20 Similarly, knockout Ztgfβ-9 mice can be used to determine
where Ztgfβ-9 is absolutely required *in vivo.* The
phenotype of knockout mice is predictive of the *in vivo*
effects of that of a Ztgfβ-9 antagonist. The human Ztgfβ-9
cDNA can be used to isolate murine Ztgfβ-9 mRNA, cDNA and
25 genomic DNA, which are subsequently used to generate
knockout or transgenic mice. These mice may be employed to
study the *Ztgfβ-9* gene and the protein encoded thereby in
an *in vivo* system, and can be used as *in vivo* models for
corresponding human diseases. Moreover, transgenic mouse
30 expression of Ztgfβ-9 antisense polynucleotides or
ribozymes directed against *Ztgfβ-9* or single chain
antibodies to Ztgfβ-9 can be used to further elucidate the
biology of Ztgfβ-9.

35   Uses

Northern blot analysis of the expression of
Ztgfβ-9 reveals that Ztgfβ-9 is highly expressed in the
brain and spinal cord. Therefore, Ztgfβ-9 may play a role
in the maintenance of spinal cord involving either glial
5   cells or neurons. This indicates that Ztgfβ-9 can be used
to treat a variety of neurodegenerative diseases such as
amyotrophic lateral sclerosis (ALS), Alzheimer's disease,
Huntington's disease, Parkinson's disease and peripheral
neuropathies, or demyelinating diseases including multiple
10 sclerosis. The tissue specificity of Ztgfβ-9 expression
suggests that Ztgfβ-9 may be a growth and/or maintenance
factor in the spinal cord and brain which can be used to
treat spinal cord, brain or peripheral nervous system
injuries. Ztgfβ-9 can also be administered to someone to
15 treat a viral infection.

The present invention also provides reagents
with significant therapeutic value. The Ztgfβ-9
polypeptide (naturally occurring or recombinant),
20 fragments thereof, antibodies and anti-idiotypic
antibodies thereto, along with compounds identified as
having binding affinity to the Ztgfβ-9 polypeptide, should
be useful in the treatment of conditions associated with
abnormal physiology or development, including abnormal
25 proliferation, e.g., cancerous conditions, or degenerative
conditions. For example, a disease or disorder associated
with abnormal expression or abnormal signaling by a
Ztgfβ-9 polypeptide should be a likely target for an
agonist or antagonist of the Ztgfβ-9 polypeptide. In
30 particular, Ztgfβ-9 can be used to treat inflammation.
Inflammation is a result of an immune response to an
infection or as an autoimmune response to a self-antigen.

Antibodies to the Ztgfβ-9 polypeptide can be
35 purified and then administered to a patient. These
reagents can be combined for therapeutic use with
additional active or inert ingredients, e.g., in

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pharmaceutically acceptable carriers or diluents along
with physiologically innocuous stabilizers and excipients.
These combinations can be sterile filtered and placed into
dosage forms as by lyophilization in dosage vials or
5   storage in stabilized aqueous preparations. This invention
also contemplates use of antibodies, binding fragments
thereof or single-chain antibodies of the antibodies
including forms which are not complement binding.

10   The quantities of reagents necessary for
effective therapy will depend upon many different factors,
including means of administration, target site,
physiological state of the patient, and other medications
administered. Thus, treatment dosages should be titrated
15   to optimize safety and efficacy. Typically, dosages used
in vitro may provide useful guidance in the amounts useful
for *in vivo* administration of these reagents. Animal
testing of effective doses for treatment of particular
disorders will provide further predictive indication of
20   human dosage. Methods for administration include oral,
intravenous, peritoneal, intramuscular, or transdermal
administration. Pharmaceutically acceptable carriers will
include water, saline or buffers to name just a few.
Dosage ranges would ordinarily be expected from 1µg to
25   1000µg per kilogram of body weight per day. However, the
doses may be higher or lower as can be determined by a
medical doctor with ordinary skill in the art. For a
complete discussion of drug formulations and dosage ranges
see *Remington's Pharmaceutical Sciences,* 17th Ed., (Mack
30   Publishing Co., Easton, Penn., 1990), and *Goodman and
Gilman's: The Pharmacological Bases of Therapeutics,* 9th Ed.
(Pergamon Press 1996).

Nucleic Acid-based Therapeutic Treatment

If a mammal has a mutated or lacks a $Ztgf\beta-9$
gene, the $Ztgf\beta-9$ gene can be introduced into the cells of
5   the mammal. In one embodiment, a gene encoding a $Ztgf\beta-9$
polypeptide is introduced *in vivo* in a viral vector. Such
vectors include an attenuated or defective DNA virus, such
as but not limited to herpes simplex virus (HSV),
papillomavirus, Epstein Barr virus (EBV), adenovirus,
10 adeno-associated virus (AAV), SV40 and the like. Defective
viruses , which entirely or almost entirely lack viral
genes, are preferred. A defective virus is not infective
after introduction into a cell. Use of defective viral
vectors allows for administration to cells in a specific,
15 localized area, without concern that the vector can infect
other cells. Examples of particular vectors include, but
are not limited to, a defective herpes virus 1 (HSV1)
vector [Kaplitt *et al.*, *Molec. Cell. Neurosci.*,*2* :320-330
(1991)], an attenuated adenovirus vector, such as the
20 vector described by Stratford-Perricaudet *et al.*, *J. Clin.
Invest.*, *90* :626-630 (1992), and a defective adeno-
associated virus vector [Samulski *et al.*, *J. Virol.*,
61:3096-3101 (1987); Samulski *et al. J. Virol.*, *63*:3822-
3828 (1989)].

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In another embodiment, the gene can be
introduced in a retroviral vector, *e.g.*, as described in
Anderson *et al.*, U.S. Patent No. 5,399,346; Mann *et al.*,
Cell, *33*:153 (1983); Temin *et al.*, U.S. Patent No.
30 4,650,764; Temin *et al.*, U.S. Patent No. 4,980,289;
Markowitz *et al.*, *J. Virol.*, *62*:1120 (1988); Temin *et al.*,
U.S. Patent No. 5,124,263; International Patent
Publication No. WO 95/07358, published March 16, 1995 by
Dougherty *et al.*; and *Blood*, *82*:845 (1993).

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Alternatively, the vector can be introduced by
lipofection *in vivo* using liposomes. Synthetic cationic

lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker [Felgner *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:7413-7417 (1987); see Mackey *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:8027-8031 (1988)]. The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides, *e.g.*, hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

It is possible to remove the cells from the body and introduce the vector as a naked DNA plasmid or by means of a viral vector and then re-implant the transformed cells into the body. Naked DNA vector for gene therapy can be introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter [see, *e.g.*, Wu *et al.*, *J. Biol. Chem.*, 267:963-967 (1992); Wu *et al.*, *J. Biol. Chem.*, 263:14621-14624 (1988)]. Techniques such as viral vector-mediated gene delivery of Ztgfβ-9 can be used to treat human diseases such as cancer, immune & autoimmune diseases, and diseases of the central and peripheral nervous system.

Ztgfβ-9 polypeptides can also be used to prepare antibodies that specifically bind to Ztgfβ-9 polypeptides. These antibodies can then be used to manufacture anti-idiotypic antibodies. As used herein, the term

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"antibodies" includes polyclonal antibodies, monoclonal antibodies, antigen-binding fragments thereof such as $F(ab')_2$ and Fab fragments, and the like, including genetically engineered antibodies. Antibodies are defined
5 to be specifically binding if they bind to a Ztgfβ-9 polypeptide with a K_a of greater than or equal to $10^7/M$ and they do not substantially bind to a polypeptide of the prior art. The affinity of a monoclonal antibody can be readily determined by one of ordinary skill in the art,
10 for example, by using Scatchard analysis.

Methods for preparing polyclonal and monoclonal antibodies are well known in the art (see for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*,
15 (Second Edition) (Cold Spring Harbor, NY, 1989); and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications* (CRC Press, Inc., Boca Raton, FL, 1982). Polyclonal antibodies can be generated by inoculating a variety of warm-blooded animals such as
20 horses, cows, goats, sheep, dogs, chickens, rabbits, mice, hamsters, guinea pigs and rats with a Ztgfβ-9 polypeptide or a fragment thereof. The immunogenicity of a Ztgfβ-9 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's
25 complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Ztgfβ-9 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length
30 molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization. A variety of
35 assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to Ztgfβ-9 polypeptides. Exemplary assays are described in detail in

Antibodies: A Laboratory Manual, Harlow and Lane (Eds.),
(Cold Spring Harbor Laboratory Press, 1988).
Representative examples of such assays include: concurrent
immunoelectrophoresis, radio-immunoassays, radio-
5   immunoprecipitations, enzyme-linked immunosorbent assays
(ELISA), dot blot assays, inhibition or competition
assays, and sandwich assays.

As used herein, the term "antibodies" includes
10   polyclonal antibodies, affinity-purified polyclonal
antibodies, monoclonal antibodies, and antigen-binding
fragments, such as $F(ab')_2$ and Fab proteolytic fragments.
Genetically engineered intact antibodies or fragments,
such as chimeric antibodies, Fv fragments, single chain
15   antibodies and the like, as well as synthetic antigen-
binding peptides and polypeptides, are also included.
Non-human antibodies may be humanized by grafting non-
human CDRs onto human framework and constant regions, or
by incorporating the entire non-human variable domains
20   (optionally "cloaking" them with a human-like surface by
replacement of exposed residues, wherein the result is a
"veneered" antibody). In some instances, humanized
antibodies may retain non-human residues within the human
variable region framework domains to enhance proper
25   binding characteristics. Through humanizing antibodies,
biological half-life may be increased, and the potential
for adverse immune reactions upon administration to humans
is reduced. Human antibodies can be generated in mice
engineered to contain the human immunoglobulin loci,
30   Vaughan, *et al. Nat. Biotech.*, *16*:535-539 (1998).

Alternative techniques for generating or
selecting antibodies useful herein include *in vitro*
exposure of lymphocytes to Ztgfβ-9 protein or peptide, and
35   selection of antibody display libraries in phage or
similar vectors (for instance, through use of immobilized

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or labeled Ztgfβ-9 protein or peptide). Genes encoding
polypeptides having potential Ztgfβ-9 polypeptide binding
domains can be obtained by screening random peptide
libraries displayed on phage (phage display) or on

5   bacteria, such as *E. coli*. Nucleotide sequences encoding
the polypeptides can be obtained in a number of ways, such
as through random mutagenesis and random polynucleotide
synthesis. These random peptide display libraries can be
used to screen for peptides which interact with a known

10   target which can be a protein or polypeptide, such as a
ligand or receptor, a biological or synthetic
macromolecule, or organic or inorganic substances.
Techniques for creating and screening such random peptide
display libraries are known in the art (Ladner et al., US

15   Patent NO. 5,223,409; Ladner et al., US Patent NO.
4,946,778; Ladner et al., US Patent NO. 5,403,484 and
Ladner et al., US Patent NO. 5,571,698) and random peptide
display libraries and kits for screening such libraries
are available commercially, for instance from Clontech

20   (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New
England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB
Biotechnology Inc. (Piscataway, NJ). Random peptide
display libraries can be screened using the Ztgfβ-9
sequences disclosed herein to identify proteins which bind

25   to Ztgfβ-9. These "binding proteins" which interact with
Ztgfβ-9 polypeptides can be used for tagging cells; for
isolating homolog polypeptides by affinity purification;
they can be directly or indirectly conjugated to drugs,
toxins, radionuclides and the like. These binding

30   proteins can also be used in analytical methods such as
for screening expression libraries and neutralizing
activity. The binding proteins can also be used for
diagnostic assays for determining circulating levels of
polypeptides; for detecting or quantitating soluble

35   polypeptides as marker of underlying pathology or disease.
These binding proteins can also act as Ztgfβ-9

"antagonists" to block Ztgfβ-9 binding and signal
transduction *in vitro* and *in vivo*.

        Antibodies can also be generated by gene
5   therapy. The animal is administered the DNA or RNA which
     encodes Ztgfβ-9 or an immunogenic fragment thereof so that
     cells of the animals are transfected with the nucleic acid
     and express the protein which in turn elicits an
     immunogenic response. Antibodies which then are produced
10   by the animal are isolated in the form of polyclonal or
     monoclonal antibodies. Antibodies to Ztgfβ-9 may be used
     for tagging cells that express the protein, for affinity
     purification, within diagnostic assays for determining
     circulating levels of soluble protein polypeptides, and as
15   antagonists to block ligand binding and signal
     transduction *in vitro* and *in vivo*.

        Radiation hybrid mapping is a somatic cell genetic
     technique developed for constructing high-resolution,
20   contiguous maps of mammalian chromosomes [Cox *et al.*,
     Science 250:245-250 (1990)]. Partial or full knowledge of
     a gene's sequence allows the designing of PCR primers
     suitable for use with chromosomal radiation hybrid mapping
     panels. Commercially available radiation hybrid mapping
25   panels which cover the entire human genome, such as the
     Stanford G3 RH Panel and the GeneBridge 4 RH Panel
     (Research Genetics, Inc., Huntsville, AL), are available.
     These panels enable rapid, PCR based, chromosomal
     localizations and ordering of genes, sequence-tagged sites
30   (STSs), and other nonpolymorphic- and polymorphic markers
     within a region of interest. This includes establishing
     directly proportional physical distances between newly
     discovered genes of interest and previously mapped
     markers. The precise knowledge of a gene's position can
35   be useful in a number of ways including: 1) determining if
     a sequence is part of an existing contig and obtaining

additional surrounding genetic sequences in various forms
such as genomic YAC-, BAC- or phage genomic clones or cDNA
clones, 2) providing a possible candidate gene for an
inheritable disease which shows linkage to the same

5   chromosomal region, and 3) for cross-referencing model
organisms such as mouse which may be beneficial in helping
to determine what function a particular gene might have.

The present invention also provides reagents

10 which will find use in diagnostic applications. For
example, the Ztgfβ-9 gene has been mapped on chromosome
13q11.2-q11. A Ztgfβ-9 nucleic acid probe could to used to
check for abnormalities on chromosome 13. For example, a
probe comprising Ztgfβ-9 DNA or RNA or a subsequence

15 thereof can be used to determine if the Ztgfβ-9 gene is
present on human chromosome 13q11.2-q11 or if a mutation
has occurred. Detectable chromosomal aberrations at the
Ztgfβ-9 gene locus include but are not limited to
aneuploidy, gene copy number changes, insertions,

20 deletions, restriction site changes and rearrangements.
Such aberrations can be detected using polynucleotides of
the present invention by employing molecular genetic
techniques, such as restriction fragment length
polymorphism (RFLP) analysis, short tandem repeat (STR)

25 analysis employing PCR techniques, and other genetic
linkage analysis techniques known in the art. Human Ztgfβ-
9 maps at the 13q11.2-q11 region. Mouse Ztgfβ-9 maps to
mouse chromosome 14 framework markers d14mit64 and dmit82
located at 22.0 and 19.5 centimorgans, respectively. The

30 19.5 cm region appears to be syntenic with the human locus
containing the gap junction genes gja3 and gjb2. See
Mignon, C. et al., Cytogenet. Cell Genet. 72: 185-186
(1996).

35   The invention is further illustrated by the
following non-limiting examples.

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Example 1.

Cloning of Ztgfβ-9

5   Human *Ztgfβ-9* was isolated from an arrayed pituitary
gland cDNA plasmid library by PCR screening using SEQ ID
NOs: 6 and 7. Thermocycler conditions were as follows: one
cycle at 94°C for 3 minutes, 35 cycles at 94°C for 30
seconds, 62°C for 20 seconds, 72°C for 30 seconds, one
10   cycle at 72°C for 5 minutes, followed by 4° C hold. The
reactions were gel electrophoresed to identify positive
pools and, in this way the library was deconvoluted to a
pool of positive clones. These were electroporated into *E.
coli* DH10B cells and plated for colony hybridization. The
15   colonies were transferred to Hybond N filters (Amersham)
and probed for positive colonies. Positive clones were
sequenced for full length Ztgfβ-9.

Sequence analysis and conceptual translation of the
20   human *Ztgfβ-9* cDNA (SEQ ID NOs: 1 and 16) predicts a
protein product that is 202 amino acid residues in length.
This protein is homologous to two members of the IL-17
family, Zcyto7, International Application No.
PCT/US98/08212, and IL-17. Ztgfβ-9 shares 27.8% amino acid
25   identity with Zcyto7 and 20.6% identity with IL-17 as
determined by the Clustal Method using Lasergene MegAlign
software. See Higgins and Sharp, *CABIOS* 5:151 (1989). In
particular, Ztgfβ-9 shares four conserved cysteines (amino
acid residues 114, 119, 167, 169 in SEQ ID NO:2) with
30   Zcyto7 and IL-17. These cysteines are predicted to be
involved in forming a cysteine-knot-like protein fold that
is related to the found in TGF-β proteins.

Example 2

35

Northern Analysis *Ztgfβ-9*

Analysis of tissue distribution was performed by
the northern blotting technique using 2 adult and 1 fetal
human brain blots, Human Multiple Tissue and Master Dot
Blots from Clontech (Palo Alto, Ca). A probe was
5 obtained by PCR using SEQ ID NOs:6 and 7. in a cDNA pool.
Thermocycler conditions were as follows: one cycle at 94°C
for 3 minutes, 35 cycles at 94°C for 10 seconds, 66°C for
20 seconds, 72°C for 30 seconds, one cycle at 72°C for 5
minutes, followed by 4°C hold. The reaction mixture was
10 electrophoresed on a preparative agarose gel and a 162 bp
fragment was gel purified using commercially available gel
purification reagents and protocol (QIAEX II Gel
Extraction Kit; Qiagen,. Inc., Santa Clarita, Ca). The
purified DNA was radioactively labeled with ^{32}P using a
15 commercially available kit (Rediprime DNA labeling
system; Amersham Corp., Arlington Heights, IL).The probe
was purified using a NUCTRAP push column(Stratagene
Cloning Systems, La Jolla, CA) . EXPRESSHYB (Clonetech,
Palo Alto, CA) solution was used for prehybridization and
20 hybridization. The hybridization solution consisted of 8
mls EXPRESSHYB, 80μl Sheared Salmon Sperm DNA (10mg/ml,5-
Prime-3 Prime, Boulder, CO), 48 μl Human Cot-1 DNA
(1mg/ml,GibcoBRL) and 18μl of radiolabeled probe.
Hybridization took place overnight at 50°C And the blots
25 were then washed in 2X SSC,0.1%SDS at RT, then2X SSC,0.1%
SDS at 60°C, followed by 0.1X SSC, 0.1% SDS wash at 60°C.
The blots were exposed overnight and developed. A major
transcript signal of was observed on MTN blots in brain
and spinal cord.
30

Master Dot blot signals were strong in all brain
tissues (adult and fetal), spinal cord, heart, skeletal
muscle, stomach, pancreas, adrenal gland, salivary gland,
liver, small intestine, bone marrow, thymus, spleen, lymph
35 node, heart, thyroid, trachea, testis, ovary and placenta.

Example 3

70

Cloning of Murine *Ztgfβ-9*

Full length sequence was obtained from a clone
5   isolated from an arrayed mouse testis cDNA/plasmid
library. The library was screened by PCR using
oligonucleotides SEQ NO:10 and SEQ ID NO:11. The library
was deconvoluted down to a positive pool of 250 clones.
E.coli DH10B cells (Gibco BRL) were transformed with this
10 pool by electroporation. The transformed culture was
titered and arrayed out to 96 wells at ~20 cells/well. The
cells were grown up in LBamp overnight at 37°C. An aliquot
of the cells were pelleted and PCR was used to identify a
positive pool. The remaining cells from a positive pool
15 were plated and colonies screened by PCR to identify a
positive clone. The clone was sequenced and contained the
putative full length sequence of murine *Ztgfβ-9*. The
sequence of murine Ztgfbeta-9 is defined by SEQ ID NOs: 8
and 9.
20

Example 4

Northern Analysis of Mouse *Ztgfβ-9*

25   Northern analysis was also carried out on Mouse MTN
and Master Dot blots (Clontech) and a Mouse Embryo blot.
A full-length murine Ztgfβ-9 cDNA clone (see cloning
section) was restriction digested with ApaI and EcoRI
following standard protocols. The reaction was gel
30 electrophoresed and the ~686bp fragment was gel purified
using the Qiaex II Gel Purification Kit (Qiagen, Valencia,
CA). The cDNA was P32-labeled using the Rediprime II
Labeling Kit (Amersham) and column purified using reagents
and protocols described earlier. Hybridization, washing,
35 and detection were carried out under conditions as
described in Example 2. A band was observed in heart,
brain, lung, liver, skeletal muscle, kidney and testis.

The Master Dot blot had strong signals in thyroid, with
fainter signals in most other tissues. Hybridization to
the Mouse Embryo blot indicated that Ztgfβ-9 was expressed
at all stages examined (embryonic days 7, 11, 15, and 17).

5

    By quantitative RT-PCR, murine Ztgfβ-9 was found to
be highly expressed in the HCL hypothalamic cell line, and
at lower levels in the GT1-1 and GT1-7 hypothalamic cell
lines and the undifferentiated P19 teratocarcinoma cell
10   line. Using quantitative RT-PCR, murine *Ztgfβ-9* was
detected in neurons of the hippocampal cerebellar and
olfactory cortex, Purkinje cells and other neuronal
populations were heavily labeled in brain sections. The
endothelium of the choroid plexus was also heavily
15   positive. In the spinal cord, labeling was confined to the
gray matter and appeared to be uniformly found in dorsal
and ventral horn neurons representing sensory and motor
neurons. Strong expression was also observed in the dorsal
root ganglia.

20

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Example 5

Antibody Production

5   Polypeptides SEQ ID NOs: 13, 14 and 15 were
synthesized and were injected into rabbits and polyclonal
anti-sera was subsequently affinity purified by column
chromatography using the cognate immunogen. Also fusion
proteins between full-length human and mouse Ztgf-β fused
10   to the C-terminus of the maltose-binding protein were
expressed in *E. coli* and purified by affinity
chromatography over an amylose resin. Purified proteins
were injected into rabbits and polyclonal anti-sera was
subsequently affinity purified by column chromatography
15   using the cognate immunogen.

Example 6

Immunocytochemistry

20

       Affinity purified polyclonal antibodies to human
Ztgfβ-9 produced according to the procedure of Example 5
were validated on normal COS cells and COS cells
transfected with a *Ztgfβ-9* mammalian cell expression
25   construct. Immunocytochemistry performed with anti-Ztgfβ-9
antibodies demonstrated Ztgfβ-9 expression in monkey brain
and spinal cord. The immunocytochemistry staining was
intracytoplasmic and observed in many large neurons and
Purkinje cells. Scattered epithelial cells in the human
30   duodenum also showed positive staining.

Example 7

Mammalian cell protein production

35   Human Ztgfβ-9 protein, both with and without a
C-terminal a Glu-Glu affinity tag [Grussenmeyer *et al.*,

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Proc. Natl. Acad. Sci. USA 82:7952-4 (1985)], was
expressed in BHK cells using an expression vector in which
Ztgfβ-9 expression is driven by the CMV immediate early
promoter, a consensus intron from the variable region of
5   mouse immunoglobulin heavy chain locus, multiple
restriction sites for insertion of coding sequences, a
stop codon and a human growth hormone terminator. The
plasmid also has an *E. coli* origin of replication, a
mammalian selectable marker expression unit having an SV40
10 promoter, enhancer and origin of replication, a DHFR gene
and the SV40 terminator and Kozac sequences at the 5' end
of the open reading frame.

        Following selection for stable cell
15 transfectants, media isolated from pools was analyzed by
western blot under reducing and non-reducing conditions
using a Ztgfβ-9 antibody or an anti-EE epitope tag
antibody. Human Ztgfβ-9 was found to migrate under
reducing conditions at 29 kDa. Since the predicted
20 molecular weight of the fully processed form of the
protein is 20.31 kDa, this suggests that the protein is
glycosylated at one or both of two potential glycosylation
sites. Under non-reducing conditions, Ztgfβ-9 protein
migrated as at 49 kDa species. These results indicate
25 that human Ztgfβ-9 is capable of forming a disulfide
cross-linked homodimer. However, co-expression of C-
terminally EE tagged human Ztgfβ-9 and untagged human
Zcyto7, followed by affinity purification using an anti-EE
tag antibody resulted in co-purification of both proteins,
30 suggesting that in addition to Ztgfβ-9 homodimers, Ztgfβ-9
and Zcyto7 can also dimerize. Interaction of Ztgfβ-9 and
Zcyto7 did not appear to be due to interchain disulfide-
bonding between the proteins. C-terminal EE tagged human
Ztgfβ-9 expressed in BHK cells was also anti-EE affinity
35 purified and its N-terminal sequence determined. The
signal cleavage site was found to occur proceeding amino
acid A23.

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Example 8

Transgenic mice

The open reading frame encoding full-length murine Ztgfβ-9 was amplified by PCR so as to introduce an optimized initiation codon and introduced into a transgenic vector in which expression of Ztgfβ-9 was regulated by the metallothionein I promoter. The transgene insert was separated from plasmid backbone by *Not*I digestion and agarose gel purification, and fertilized ova from matings of B6C3F1Tac mice were microinjected and implanted into pseudopregnant females. Founders were identified by PCR on genomic tail DNA (DNAeasy 96 kit; Qiagen). Transgenic lines were initiated by breeding founders with C57BL/6Tac mice. Animal protocols used in this study were approved by the ZymoGenetics Institutional Animal Care and Use Committee. From 49 progeny born only 8% were found to be transgenic (compared to an average of 20% observed for a variety of other cDNAs driven by the same promoter), suggesting that high expression of murine Ztgfβ-9 may be embryonic lethal. Consistent with this, of the four founders identified, all expressed only low levels of Ztgfβ-9 mRNA in the liver. A fifth founder died at birth and interestingly, this animal expressed very high levels of Ztgfβ-9 mRNA in the liver (8500 copies/cell). Histopathological analysis of this animal identified severe apoptosis of the thymus and complete devaculization of brown fat. The expressing males were bred with wild-type females. One founder was capable of germline transmission, however all transgenic progeny of this founder either died at birth or were runted and died soon after weaning. Analysis of these animals identified a

variety of phenotypes, including severe thymic apoptosis,
devaculization of brown fat, liver hepatitis, and low
lymphocyte peripheral blood cell numbers. These results
indicate that Ztgfβ-9, its agonists and antagonists, and
5   antibodies to Ztgfβ-9 will be useful in regulating immune
cells, adipogenesis, and liver cells.

Example 9

10   Chromosomal position

     Human Ztgfβ-9 was mapped to chromosome 13q11.2 on two
radiation hybrid panels. The mouse Ztgfb9 gene links to
murine chromosome 14 framework markers d14mit64 and dmit82
15   located at 22.0 and 19.5 centimorgans, respectively.

Example 10

       Inhibition of Adenovirus Growth by Ztgfβ-9
20
     The human and murine Ztgfβ-9 coding region were
cloned into the adenovirus shuttle vector and recombined
to generated the recombinant adenoviral genome.
Transfection of *Ztgfβ-9* adenoviral genomes into 293A cells
25   resulted in very small viral plaques (1 or 2 plaques per
transfection which is low). These plaques did not expand
in size. Normally plaques expand greatly in size over a
period of 1-2 days as the virus replicates. The small
plaques were harvested and we tried to expand the virus by
30   infecting 293A cells. Infected monolayers again exhibited
very low numbers of plaques and the plaques were small in
size. These plaques did not expand in size over time.
After 2-3 rounds of attempting to amplify the virus,
eventually we did obtain a rapidly growing virus
35   population. The virus that results from this
amplification still contains the Ztgfβ-9 sequences.
However, infection of cell by these viruses did not result

in protein production. The initial behavior of both the mouse and human *Ztgfβ-9*-containing viruses is unlike we have seen with any other cDNA. Clearly, virus replication was inhibited.

5

CLAIMS

We claim:

      1.   An isolated polynucleotide which encodes a
polypeptide, said polypeptide being comprised of an amino acid
sequence, said amino acid sequence being at least 90%
identical to one or more of the amino acid sequences selected
from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID
NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13,
SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID
NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

      2.   An isolated polynucleotide of claim 1 wherein
the polynucleotide encodes a polypeptide containing an amino
acid sequence selected from the group consisting of SEQ ID
NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, SEQ
ID NO:12, SEQ ID NO:17 and SEQ ID NO:18.

      3.   An isolated polynucleotide of claim 1 wherein
the polynucleotide encodes a polypeptide comprised of an amino
acid sequence selected from the group consisting of SEQ ID
NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:13, SEQ
ID NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID
NO:19, SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

      4.   An isolated polynucleotide of claim 3 wherein
the polynucleotide encodes a polypeptide comprised of an amino
acid sequence selected from the group consisting of SEQ ID
NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

      5.   An isolated polynucleotide of claim 1 wherein
said polynucleotide is selected from the group consisting of
SEQ ID NO:1 and SEQ ID NO:9.

      6.   An isolated polypeptide comprised of an amino
acid sequence, said amino acid sequence being at least 90%

identical to an amino acid sequence selected from the group
consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID
NO:5, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14,
SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID
NO:20, SEQ ID NO:21 and SEQ ID NO:22.

         7.   An isolated polypeptide of claim 6 comprised of
an amino acid sequence selected from the group consisting of
SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID
NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15,
SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID
NO:21 and SEQ ID NO:22.

         8.   An isolated polypeptide of claim 6 wherein the
amino acid sequence is selected from the group consisting of
SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID
NO:9 and SEQ ID NO:12.

         9.   An isolated polypeptide of claim 6 wherein the
amino acid sequence is selected from the group consisting of
SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and SEQ ID NO:5.

         10.   An antibody which specifically binds to a
polypeptide comprised of an amino acid sequence selected from
the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4,
SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:13, SEQ ID
NO:14, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19,
SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22.

         11.   An anti-idiotypic antibody which binds to an
antibody of claim 12.

SEQUENCE LISTING

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        1201 Eastlake Avenue East
        Seattle, Washington 98102
        United States of America

<120> Mammalian Transforming Growth Factor
    Beta - 9

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2

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Phe Arg Pro Pro Thr Asn Leu Arg Ser Val Ser Pro Trp Ala Tyr Arg
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Ile Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu Pro Glu Ala Tyr
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    <212> DNA
    <213> Homo sapiens


    <400> 6
ccgggtcgta ggagattctg tag                                    23


    <210> 7
    <211> 22
    <212> DNA
    <213> Homo sapiens


    <400> 7
gcgtgctcag tgccttccac ca                                     22


    <210> 8
    <211> 1221
    <212> DNA
    <213> Mus musculis


    <220>
    <221> CDS
    <222> (79)...(693)


    <400> 8
gggtgtcgcc cttatttact tcgcagaaga gccttcagcc ccctcctaa caagtctgga      60
aagcatcacg gcgacgcg atg ttg ggg aca ctg gtc tgg atg ctc gcg gtc     111
                     Met Leu Gly Thr Leu Val Trp Met Leu Ala Val
                      1           5                   10


ggc ttc ctg ctg gca ctg gcg ccg ggc cgc gcg gcg ggc gcg ctg agg   159
Gly Phe Leu Leu Ala Leu Ala Pro Gly Arg Ala Ala Gly Ala Leu Arg
         15                  20                  25


acc ggg agg cgc ccg gcg cgg ccg cgg gac tgc gcg gac cgg ccg gag   207
Thr Gly Arg Arg Pro Ala Arg Pro Arg Asp Cys Ala Asp Arg Pro Glu
     30                  35                  40


gag ctc ctg gag cag ctg tac ggg cgg ctg gcg gcc ggc gtg ctc agc   255
Glu Leu Leu Glu Gln Leu Tyr Gly Arg Leu Ala Ala Gly Val Leu Ser
```

```
        45                    50                    55

gcc ttc cac cac acg ctg cag ctc ggg ccg cgc gag cag gcg cgc aat    303
Ala Phe His His Thr Leu Gln Leu Gly Pro Arg Glu Gln Ala Arg Asn
 60                  65                  70                  75

gcc agc tgc ccg gcc ggg ggc agg gcc gcc gac cgc cgc ttc cgg cca    351
Ala Ser Cys Pro Ala Gly Gly Arg Ala Ala Asp Arg Arg Phe Arg Pro
                80                  85                  90

ccc acc aac ctg cgc agc gtg tcg ccc tgg gcg tac agg att tcc tac    399
Pro Thr Asn Leu Arg Ser Val Ser Pro Trp Ala Tyr Arg Ile Ser Tyr
                95                  100                 105

gac cct gct cgc ttt ccg agg tac ctg ccc gaa gcc tac tgc ctg tgc    447
Asp Pro Ala Arg Phe Pro Arg Tyr Leu Pro Glu Ala Tyr Cys Leu Cys
            110                 115                 120

cga ggc tgc ctg acc ggg ctc tac ggg gag gag gac ttc cgc ttt cgc    495
Arg Gly Cys Leu Thr Gly Leu Tyr Gly Glu Glu Asp Phe Arg Phe Arg
            125                 130                 135

agc aca ccc gtc ttc tct cca gcc gtg gtg ctg cgg cgc aca gcg gcc    543
Ser Thr Pro Val Phe Ser Pro Ala Val Val Leu Arg Arg Thr Ala Ala
140                 145                 150                 155

tgc gcg ggc ggc cgc tct gtg tac gcc gaa cac tac atc acc atc ccg    591
Cys Ala Gly Gly Arg Ser Val Tyr Ala Glu His Tyr Ile Thr Ile Pro
                160                 165                 170

gtg ggc tgc acc tgc gtg ccc gag ccg gac aag tcc gcg gac agt gcg    639
Val Gly Cys Thr Cys Val Pro Glu Pro Asp Lys Ser Ala Asp Ser Ala
            175                 180                 185

aac tcc agc atg gac aag ctg ctg ctg ggg ccc gcc gac agg cct gcg    687
Asn Ser Ser Met Asp Lys Leu Leu Leu Gly Pro Ala Asp Arg Pro Ala
        190                 195                 200

ggg cgc tgatgccggg gactgcccgc catggcccag cttcctgcat gcatcaggtc    743
Gly Arg
    205

ccctggccct gacaaaaccc accccatgat ccctggccgc tgcctaattt ttccaaaagg    803
acagctacat aagctttaaa tatatttttc aaagtagaca ctacatatct acaactattt    863
tgaatagtgg cagaaactat tttcatatta gtaatttaga gcaagcatgt tgtttttaaa    923
```

8

```
cttctttgat atacaagcac atcacacaca tcccgttttc ctctagtagg attcttgagt      983
gcataattgt agtgctcaga tgaacttcct tctgctgcac tgtgccctgt ccctgagtct     1043
ctcctgtggc ccaagcttac taaggtgata atgagtgctc cggatctggg cacctaaggt     1103
ctccaggtcc ctggagaggg agggatgtgg gggggctaga accaagcgcc cctttgttct     1163
ttagcttatg gatggtctta actttataaa gattaaagtt tttggtgtta ttctttca      1221
```

        <210> 9.
        <211> 205
        <212> PRT
        <213> Mus musculis

        <400> 9

```
Met Leu Gly Thr Leu Val Trp Met Leu Ala Val Gly Phe Leu Leu Ala
 1               5                  10                  15
Leu Ala Pro Gly Arg Ala Ala Gly Ala Leu Arg Thr Gly Arg Arg Pro
            20                  25                  30
Ala Arg Pro Arg Asp Cys Ala Asp Arg Pro Glu Glu Leu Leu Glu Gln
        35                  40                  45
Leu Tyr Gly Arg Leu Ala Ala Gly Val Leu Ser Ala Phe His His Thr
    50                  55                  60
Leu Gln Leu Gly Pro Arg Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala
65                  70                  75                  80
Gly Gly Arg Ala Ala Asp Arg Arg Phe Arg Pro Thr Asn Leu Arg
            85                  90                  95
Ser Val Ser Pro Trp Ala Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Phe
            100                 105                 110
Pro Arg Tyr Leu Pro Glu Ala Tyr Cys Leu Cys Arg Gly Cys Leu Thr
            115                 120                 125
Gly Leu Tyr Gly Glu Glu Asp Phe Arg Phe Arg Ser Thr Pro Val Phe
    130                 135                 140
Ser Pro Ala Val Val Leu Arg Arg Thr Ala Ala Cys Ala Gly Gly Arg
145                 150                 155                 160
Ser Val Tyr Ala Glu His Tyr Ile Thr Ile Pro Val Gly Cys Thr Cys
                165                 170                 175
Val Pro Glu Pro Asp Lys Ser Ala Asp Ser Ala Asn Ser Ser Met Asp
            180                 185                 190
Lys Leu Leu Leu Gly Pro Ala Asp Arg Pro Ala Gly Arg
    195                 200                 205
```

        <210> 10
        <211> 23
        <212> DNA
        <213> Homo sapiens

        <400> 10

gatcatgggg tgggttttgt cag   23

        <210> 11
        <211> 22
        <212> DNA
        <213> Homo sapiens

        <400> 11
gaggacttcc gctttcgcaa ca   22

        <210> 12
        <211> 183
        <212> PRT
        <213> Mus musculis

        <400> 12
Ala Gly Ala Leu Arg Thr Gly Arg Arg Pro Ala Arg Pro Arg Asp Cys
1   5   10   15
Ala Asp Arg Pro Glu Glu Leu Leu Glu Gln Leu Tyr Gly Arg Leu Ala
            20   25   30
Ala Gly Val Leu Ser Ala Phe His His Thr Leu Gln Leu Gly Pro Arg
        35   40   45
Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala Gly Gly Arg Ala Ala Asp
    50   55   60
Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser Val Ser Pro Trp Ala
65   70   75   80
Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Phe Pro Arg Tyr Leu Pro Glu
            85   90   95
Ala Tyr Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Tyr Gly Glu Glu
        100   105   110
Asp Phe Arg Phe Arg Ser Thr Pro Val Phe Ser Pro Ala Val Val Leu
        115   120   125
Arg Arg Thr Ala Ala Cys Ala Gly Gly Arg Ser Val Tyr Ala Glu His
        130   135   140
Tyr Ile Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Asp Lys
145   150   155   160
Ser Ala Asp Ser Ala Asn Ser Ser Met Asp Lys Leu Leu Leu Gly Pro
            165   170   175
Ala Asp Arg Pro Ala Gly Arg
            180

        <210> 13
        <211> 30
        <212> PRT
        <213> Homo sapiens

<400> 13
Gln Leu Gly Pro Arg Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala Gly
1   5   10   15
Gly Arg Pro Ala Asp Arg Arg Phe Arg Pro Pro Thr Asn Leu
            20   25   30

<210> 14
<211> 21
<212> PRT
<213> Homo sapiens

<400> 14
Gly Glu Glu Asp Val Arg Phe Arg Ser Ala Pro Val Tyr Met Pro Thr
1   5   10   15
Val Val Leu Arg Cys
            20

<210> 15
<211> 34
<212> PRT
<213> Homo sapiens

<400> 15
Cys Val Pro Glu Pro Glu Lys Asp Ala Asp Ser Ile Asn Ser Ser Ile
1   5   10   15
Asp Lys Gln Gly Ala Lys Leu Leu Leu Gly Pro Asn Asp Ala Pro Ala
            20   25   30
Gly Pro

<210> 16
<211> 2361
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (572)...(1202)

<400> 16
gaattcggca cgagggtcag ggaagtattc agtgctttgt tgtagagttg ttggatagag   60
gcacaggatc atttcatgtt gttgaggaga aaggagcaac agcctcctcc caccttatta   120
aaaatagaga tttaaaaaaa cctctaattt cctcgaagta cagaatctca agaggtagct   180
ctaaggagaa tccctctggg tttgagcgca ttcctcttcc aggggggccta ttcttggact   240

```
gctttcctta atagagaaat ctctctgagc caaaatcggc ctcccccaat tccatcctgt      300
cggccccact tttctgctcc ggagacttcc aagccagtcc ccactcctcc ttcagccagt      360
cgggcccgca cccgcgcccg gcagggccag ccctctcctc ctcctgcgtg gcgcagcaca      420
ggccctgagc gcgcgacccc aggccctggg cgccccgccg catgctcgcg gctggaagcc      480
ccagtttgcg tggcccttcg ggttattccg ctcaagagcc gccgcgtcgc cccatctcgg      540
cgcgaatctg aaagcgcttt cggggggagaa g atg ttg ggg gca ctg gtc tgg       592
                                      Met Leu Gly Ala Leu Val Trp
                                        1             5
```

```
atg ctg gta gcc ggc ttc ctg ctg gcg ctg ccg ccg agc tgg gcc gcg      640
Met Leu Val Ala Gly Phe Leu Leu Ala Leu Pro Pro Ser Trp Ala Ala
         10              15              20
```

```
ggc gcc ccg agg gcg ggc agg cgc ccc gcg cgg ccg cgg ggc tgc gcg      688
Gly Ala Pro Arg Ala Gly Arg Arg Pro Ala Arg Pro Arg Gly Cys Ala
     25              30              35
```

```
gac cgg ccg gag gag cta ctg gag cag ctg tac ggg cgc ctg gcg gcc      736
Asp Arg Pro Glu Glu Leu Leu Glu Gln Leu Tyr Gly Arg Leu Ala Ala
 40              45              50              55
```

```
ggc gtg ctc agt gcc ttc cac cac acg ctg cag ctg ggg ccg cgt gag      784
Gly Val Leu Ser Ala Phe His His Thr Leu Gln Leu Gly Pro Arg Glu
             60              65              70
```

```
cag gcg cgc aac gcg agc tgc ccg gca ggg ggc agg ccc gcc gac cgc      832
Gln Ala Arg Asn Ala Ser Cys Pro Ala Gly Gly Arg Pro Ala Asp Arg
             75              80              85
```

```
cgc ttc cgg ccg ccc acc aac ctg cgc agc gtg tcg ccc tgg gcc tac      880
Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser Val Ser Pro Trp Ala Tyr
         90              95              100
```

```
aga atc tcc tac gac ccg gcg agg tac ccc agg tac ctg cct gaa gcc      928
Arg Ile Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu Pro Glu Ala
     105             110             115
```

```
tac tgc ctg tgc cgg ggc tgc ctg acc ggg ctg ttc ggc gag gag gac      976
Tyr Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Phe Gly Glu Glu Asp
120             125             130             135
```

```
gtg cgc ttc cgc agc gcc cct gtc tac atg ccc acc gtc gtc ctg cgc     1024
Val Arg Phe Arg Ser Ala Pro Val Tyr Met Pro Thr Val Val Leu Arg
             140             145             150
```

```
cgc acc ccc gcc tgc gcc ggc ggc cgt tcc gtc tac acc gag gcc tac        1072
Arg Thr Pro Ala Cys Ala Gly Gly Arg Ser Val Tyr Thr Glu Ala Tyr
            155             160             165

gtc acc atc ccc gtg ggc tgc acc tgc gtc ccc gag ccg gag aag gac        1120
Val Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Glu Lys Asp
        170             175             180

gca gac agc atc aac tcc agc atc gac aaa cag ggc gcc aag ctc ctg        1168
Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu Leu
        185             190             195

ctg ggc ccc aac gac gcg ccc gct ggc ccc tga g gccggtcctg             1212
Leu Gly Pro Asn Asp Ala Pro Ala Gly Pro  *
200             205
```

```
ccccgggagg tctccccggc ccgcatcccg aggcgcccaa gctggagccg cctggagggc     1272
tcggtcggcg acctctgaag agagtgcacc gagcaaacca agtgccggag caccagcgcc     1332
gcctttccat ggagactcgt aagcagcttc atctgacacg ggaatccctg gcttgctttt     1392
agctacaagc aagcagcgtg gctggaagct gatgggaaac gacccggcac gggcatcctg     1452
tgtgcggccc gcatggaggg tttggaaaag ttcacggagg ctccctgagg agcctctcag     1512
atcggctgct gcgggtgcag ggcgtgactc accgctgggt gcttgccaaa gagataggga     1572
cgcatatgct ttttaaagca atctaaaaat aataataagt atagcgacta tatacctact     1632
tttaaaatca actgtttтga atagaggcag agctatttta tattatcaaa tgagagctac     1692
tctgttacat ttcttaacat ataaacatcg ttttttactt cttctggtag aatttttttaa    1752
agcataattg gaatccttgg ataaattttg tagctggtac actctggcct gggtctctga     1812
attcagcctg tcaccgatgg ctgactgatg aaatggacac gtctcatctg acccactctt     1872
ccttccactg aaggtcttca cgggcctcca ggtggaccaa agggatgcac aggcggctcg     1932
catgccccag ggccagctaa gagttccaaa gatctcagat ttggttttag tcatgaatac     1992
ataaacagtc tcaaactcgc acaatttttt ccccctttg aaagccactg gggccaattt      2052
gtggttaaga ggtggtgaga taagaagtgg aacgtgacat ctttgccagt tgtcagaaga     2112
atccaagcag gtattggctt agttgtaagg ctttaggat caggccgaat atgaggacaa      2172
agtgggccac gttagcatct gcagagatca atctggaggc ttctgtttct gcattctgcc     2232
acgagagcta ggtccttgat cttttcttta gattgaaagt ctgtctctga acacaattat     2292
ttgtaaaagt tagaagttct tttttaaatc attaaaagag cttgctgaa aaaaaaaaaa       2352
aaaaaaaaa                                                            2361
```

```
        <210> 17
        <211> 209
        <212> PRT
        <213> Homo sapiens

        <400> 17
Met Leu Gly Ala Leu Val Trp Met Leu Val Ala Gly Phe Leu Leu Ala
 1               5               10              15
```

Leu Pro Pro Ser Trp Ala Ala Gly Ala Pro Arg Ala Gly Arg Arg Pro
            20   25   30
Ala Arg Pro Arg Gly Cys Ala Asp Arg Pro Glu Glu Leu Leu Glu Gln
            35   40   45
Leu Tyr Gly Arg Leu Ala Ala Gly Val Leu Ser Ala Phe His His Thr
        50   55   60
Leu Gln Leu Gly Pro Arg Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala
65   70   75   80
Gly Gly Arg Pro Ala Asp Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg
                85   90   95
Ser Val Ser Pro Trp Ala Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Tyr
            100   105   110
Pro Arg Tyr Leu Pro Glu Ala Tyr Cys Leu Cys Arg Gly Cys Leu Thr
        115   120   125
Gly Leu Phe Gly Glu Glu Asp Val Arg Phe Arg Ser Ala Pro Val Tyr
    130   135   140
Met Pro Thr Val Val Leu Arg Arg Thr Pro Ala Cys Ala Gly Gly Arg
145   150   155   160
Ser Val Tyr Thr Glu Ala Tyr Val Thr Ile Pro Val Gly Cys Thr Cys
            165   170   175
Val Pro Glu Pro Glu Lys Asp Ala Asp Ser Ile Asn Ser Ser Ile Asp
        180   185   190
Lys Gln Gly Ala Lys Leu Leu Leu Gly Pro Asn Asp Ala Pro Ala Gly
    195   200   205
Pro

        <210> 18
        <211> 187
        <212> PRT
        <213> Homo sapiens

        <400> 18
Ala Gly Ala Pro Arg Ala Gly Arg Arg Pro Ala Arg Pro Arg Gly Cys
1   5   10   15
Ala Asp Arg Pro Glu Glu Leu Leu Glu Gln Leu Tyr Gly Arg Leu Ala
            20   25   30
Ala Gly Val Leu Ser Ala Phe His His Thr Leu Gln Leu Gly Pro Arg
        35   40   45
Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala Gly Gly Arg Pro Ala Asp
    50   55   60
Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser Val Ser Pro Trp Ala
65   70   75   80
Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu Pro Glu
            85   90   95

```
Ala Tyr Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Phe Gly Glu Glu
            100                 105                 110
Asp Val Arg Phe Arg Ser Ala Pro Val Tyr Met Pro Thr Val Val Leu
            115                 120                 125
Arg Arg Thr Pro Ala Cys Ala Gly Gly Arg Ser Val Tyr Thr Glu Ala
        130                 135                 140
Tyr Val Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Glu Lys
145                 150                 155                 160
Asp Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu
            165                 170                 175
Leu Leu Gly Pro Asn Asp Ala Pro Ala Gly Pro
            180                 185


        <210> 19
        <211> 54
        <212> PRT
        <213> Homo sapiens


        <400> 19
Gln Leu Gly Pro Arg Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala Gly
1               5                   10                  15
Gly Arg Pro Ala Asp Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser
            20                  25                  30
Val Ser Pro Trp Ala Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Tyr Pro
            35                  40                  45
Arg Tyr Leu Pro Glu Ala
    50


        <210> 20
        <211> 16
        <212> PRT
        <213> Homo sapiens


        <400> 20
Arg Pro Ala Asp Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser Val
1               5                   10                  15


        <210> 21
        <211> 57
        <212> PRT
        <213> Homo sapiens


        <400> 21
Arg Arg Thr Pro Ala Cys Ala Gly Gly Arg Ser Val Tyr Thr Glu Ala
1               5                   10                  15
```

Tyr Val Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Glu Lys
          20   25   30
Asp Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu
          35   40   45
Leu Leu Gly Pro Asn Asp Ala Pro Ala
       50   55

      <210> 22
      <211> 14
      <212> PRT
      <213> Homo sapiens

      <400> 22
Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu Pro Glu Ala
1   5   10

```
Ala Tyr Cys Leu Cys Arg Gly Cys Leu Thr Gly Leu Phe Gly Glu Glu
            100                     105             110
Asp Val Arg Phe Arg Ser Ala Pro Val Tyr Met Pro Thr Val Val Leu
        115                 120             125
Arg Arg Thr Pro Ala Cys Ala Gly Gly Arg Ser Val Tyr Thr Glu Ala
    130             135             140
Tyr Val Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Glu Lys
145             150                 155                 160
Asp Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu
            165                 170             175
Leu Leu Gly Pro Asn Asp Ala Pro Ala Gly Pro
            180             185
```

```
        <210> 19
        <211> 54
        <212> PRT
        <213> Homo sapiens


        <400> 19
Gln Leu Gly Pro Arg Glu Gln Ala Arg Asn Ala Ser Cys Pro Ala Gly
1               5                   10              15
Gly Arg Pro Ala Asp Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser
        20                  25              30
Val Ser Pro Trp Ala Tyr Arg Ile Ser Tyr Asp Pro Ala Arg Tyr Pro
        35                  40              45
Arg Tyr Leu Pro Glu Ala
    50
```

```
        <210> 20
        <211> 16
        <212> PRT
        <213> Homo sapiens


        <400> 20
Arg Pro Ala Asp Arg Arg Phe Arg Pro Pro Thr Asn Leu Arg Ser Val
1               5                   10                  15
```

```
        <210> 21
        <211> 57
        <212> PRT
        <213> Homo sapiens


        <400> 21
Arg Arg Thr Pro Ala Cys Ala Gly Gly Arg Ser Val Tyr Thr Glu Ala
1               5                   10                  15
```

Tyr Val Thr Ile Pro Val Gly Cys Thr Cys Val Pro Glu Pro Glu Lys
                20   25   30
Asp Ala Asp Ser Ile Asn Ser Ser Ile Asp Lys Gln Gly Ala Lys Leu
            35   40   45
Leu Leu Gly Pro Asn Asp Ala Pro Ala
    50   55

        <210> 22
        <211> 14
        <212> PRT
        <213> Homo sapiens

        <400> 22
Ser Tyr Asp Pro Ala Arg Tyr Pro Arg Tyr Leu Pro Glu Ala
1   5   10